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EFFECTS OF THE PARALYTIC SHELL-FISH POISON ON NERVE CELLS

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Studies in progress for several years at this laboratory have dealt with many divergent phases of mussel poisoning (Meyer and collaborators,[1] Sommer and Meyer[2]). The peak of the season for mussel poison in the vicinity of San Francisco parallels the relative increase in numbers of a marine dinoflagellate (Sommer, Whedon, Kofoid and Stohler[3]). The toxic factor acts, after being absorbed from the gastrointestinal tract, as a depressor of respiration and inhibitor of cardiac action and also influences vasomotor activities centrally (Prinzmetal, Sommer and Leake[4]). More recently Kellaway[5] ascribed the action of mussel poison as prepared by Sommer to a "neurotoxin with both central and peripheral actions."

The effects of various poisons and toxins on the cells of the central nervous system are problems which have yielded results of interest, although many controversial views regarding the action of the substances studied on cytologic constituents have arisen. Not of least importance are the methods utilized for the detection of the changes within the nerve cells. These changes may be revealed by means of modern cytologic methods. Through the use of such methods attention was directed to the alterations which might occur in the central nervous systems of animals experimentally poisoned. Since the observations at autopsy

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3. Sommer, H.; Whedon, W. F.; Kofoid, C. A., and Stohler, R.: The Relation of Plankton to Paralytic Shellfish Poison, Arch. Path., to be published.

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in human victims of mussel poisoning had yielded nothing significant, it was thought that experimentation on animals might throw some light on the problem.

MATERIAL AND METHODS

Laboratory animals of several different species were used for this study. Fifteen animals were given the poison by subcutaneous injections. Nine of these were guinea-pigs, ranging in weight from 218 to 312 Gm. Three were rabbits, weighing from 995 to 1,015 Gm. Three were 10 day old kittens, with weights of from 195 to 220 Gm. Nerve tissues and organs from five guinea-pigs, two rabbits and one kitten served as controls. The lethal dose of the poison was determined on the basis of the body weight of each animal as compared with the 20 Gm. mouse

Summary of Experiments

Animal	Weight, Gm.	Subcutaneous Injections	Time Between Injections	Total Time of Experiment	Total Amount of Poison, M. D.*	Comment
Guinea-pig						
1	247	2	24 hours	48 hours	20	Killed 1 hour after last injection of 10 M. D.
2	234	1		26 minutes	10	Died
3	218	1		17 minutes	10	Died
4	312	3	1 hour	3¾ hours	12	Died 45 minutes after last injection of 4 M. D.
5	243	3	1 hour	3 hours	12	Died 4 minutes after last injection of 4 M. D.
6	255	3	1 hour	3½ hours	12	Died 30 minutes after last injection of 4 M. D.
7	298	2	12 hours	24 hours	22	Died 13 minutes after last injection of 18 M. D.
8	320	3	24 hours	72 hours	9	Died 14 minutes after last injection of 5 M. D.
9	260	3	24 hours	72 hours	9	Died 7 minutes after last injection of 5 M. D.
Rabbit						
10	1,000	5	1-5 days	20 days	71	Died 12 minutes after last injection of 24 M. D.
11	1,015	9	1-7 days	24 days	401	Died 26 minutes after last injection of 100 M. D.
12	995	1		11 minutes	35	Died
Kitten						
13	220	1		8 minutes	11	Died
14	220	1		11 minutes	5	Died
15	195	2	24 hours	48 hours	6½	Died 21 minutes after last injection of 4 M. D.

* M. D., or mouse dose, means the amount of poison necessary to kill a mouse weighing 20 Gm. in fifteen minutes.

(1 mouse dose has been established as the amount of poison necessary to kill a mouse weighing 20 Gm. in fifteen minutes). In the table are to be found data on the following points: The species and weight of each animal; the amount of poison injected subcutaneously; the number of injections, and the length of time each animal survived. Five succumbed to the poison within from eight to twenty-six minutes. The remaining animals were not given lethal doses of poison but were either killed later or died from an additional injection of the poison after several hours or days. The animals were killed by decapitation to avoid the effects of an anesthetic on the cytologic constituents.

Small pieces from the different regions of the central nervous system of each animal were fixed in various solutions.[6] Sections from 2 to 5 microns in thickness

6. The formulas of all fixatives and stains used in this study are to be found in C. E. McClung's "Handbook of Microscopical Technique," New York, Paul B. Hoeber, Inc., 1929.

were made of each block. Zenker's solution was used for the demonstration of Nissl substance and general histologic changes after staining with Giemsa's stain. Material fixed in Regaud's solution was stained with aniline acid fuchsin and methyl green for mitochondria. Preparations of the Golgi apparatus were made by the use of Kolatchev's osmic acid mixture. Bielschowsky's method was used for the demonstration of neurofibrils. The Feulgen reaction was studied after sectioning and applying the reagents to tissue previously fixed in saturated aqueous mercury bichloride, 1 part, and absolute alcohol, 1 part. Tissues for incineration by the method of Policard as modified by Scott[7] were fixed in absolute alcohol and solution of formaldehyde U. S. P. (9 parts to 1 part). Pieces from organs of each animal were treated with Zenker's acetic acid solution and the sections stained by Giemsa's method. Mitochondrial changes in organs were studied after Regaud fixation and aniline acid fuchsin and methyl green staining.[7]

OBSERVATIONS

On macroscopic examination of the central nervous systems of the poisoned animal there were softening and edema of the brain with moderate congestion of surface vessels. There was no evidence of hemorrhages into the spinal cord or brain. The lungs revealed large and small areas of hemorrhage, and the abdominal viscera were congested with blood. Microscopic examination of the organs revealed the usual changes accompanying agonal death. Hemorrhages into the medulla of the adrenal and the alveoli of the lung were of common occurrence.

The Nissl substance of the nerve cells in the spinal cord and medulla was altered only if the animal had received the poison over a considerable period of time. The large nerve cells of the cerebrum in every animal remained normal in appearance for cytologic constituents. In figure 1, *A*, *B* and *C* illustrate the gradations in chromatolysis of the large cells of the ventral horns of the spinal cord. The number of cells showing chromatolysis was directly dependent on the amount of poison injected and the length of time the animal was poisoned. Rabbit 11, for example, received poison in large doses over a period of twenty-four days (fig. 1 *C*).

Mitochondria were apparently resistant to the action of the poison. They appeared normal in the various regions of the brain and cord, as no qualitative or quantitative alterations were discernible. Preparations of renal tissue revealed changes in the mitochondria of the convoluted tubules. The mitochondria in certain regions of the convoluted tubules of chronically poisoned animals were spherical, and the cells containing them were considerably swollen. Otherwise no significant alterations were found in the various organs.

The Golgi apparatus as demonstrated by the osmic acid method was unchanged in any of the cells of the spinal cord or brain. Of interest, however, were the changes in its appearance in the ganglion cells of the cord in kittens and guinea-pigs. These animals received several doses of the poison. Figure 2 serves to illustrate the changes. In *A*, at the left, is a cell containing a normal Golgi apparatus. The adjacent cell shows clumping as well as dispersion of the apparatus and peripheral displacement of the nucleus. Three similarly altered cells are illustrated in *B*. The substance and position of the nuclei showed no microscopic changes with the exception of the nuclei of the ganglion cells of the spinal cord. The eccentric position of the nucleus and the alterations in the Golgi apparatus occurred in the same cell. The Feulgen reaction was identical for normal and poisoned animals.

The reaction of neurofibrils to the poison was consistent with that of other constituents, such as the Nissl substance. The neurofibrils in the peripheries of

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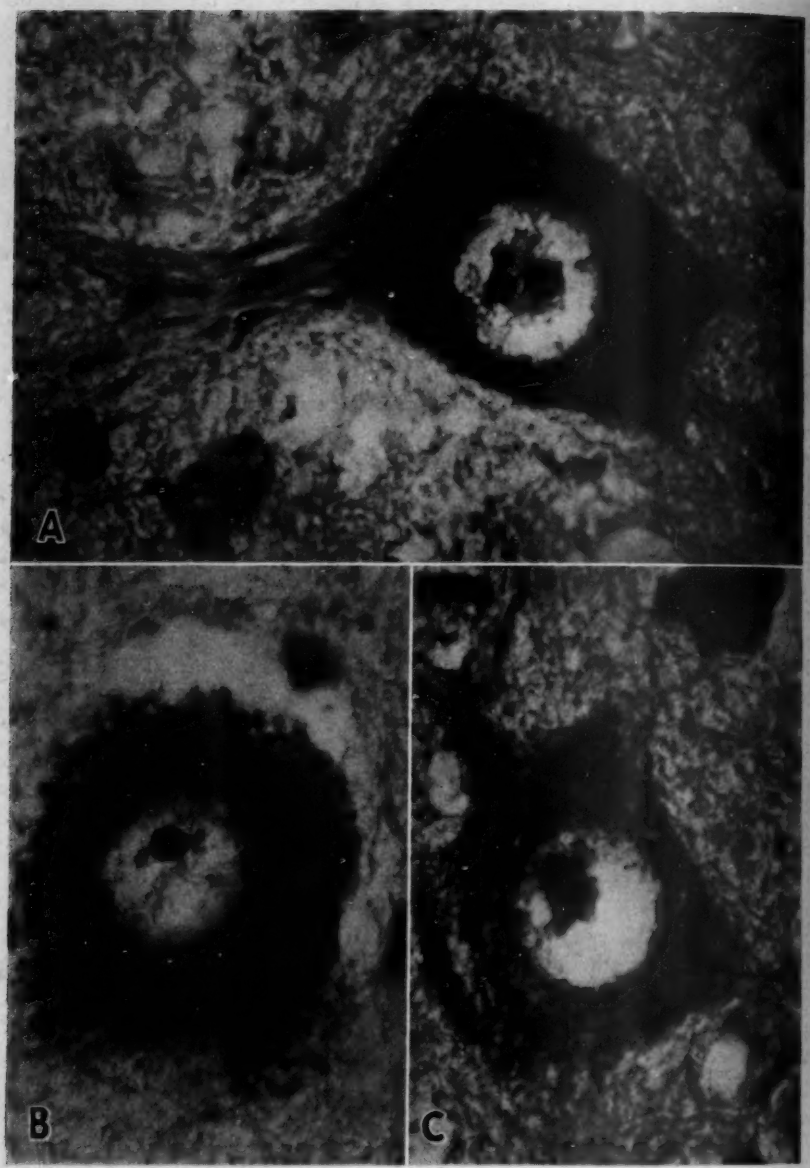

Fig. 1.—*A* shows the distribution of the Nissl substance in a large cell of the ventral horn of the spinal cord of a rabbit—a normal cell; × 1,166. *B* shows a cell of the same type from rabbit 11; × 1,166. The cell reveals slight peripheral chromatolysis as compared with the cell shown in *A*. *C* shows another cell of the same type from rabbit 11; × 1,166. There is considerable loss of Nissl substance, and it is unequally distributed. This animal received the poison over a period of twenty-four days.

Fig. 2.—*A* shows the Golgi apparatus in two spinal ganglion cells of kitten 15, which received poison over a period of two days; × 1,566. In the cell to the right, note the eccentric position of the nucleus and the tendency toward both slight dispersion and condensation of the Golgi apparatus. The cell to the left appears normal. *B* presents a group of small and medium-sized ganglion cells of the spinal cord of kitten 15, illustrating the eccentric position of the nucleus and the appearance of the Golgi apparatus; × 1,566.

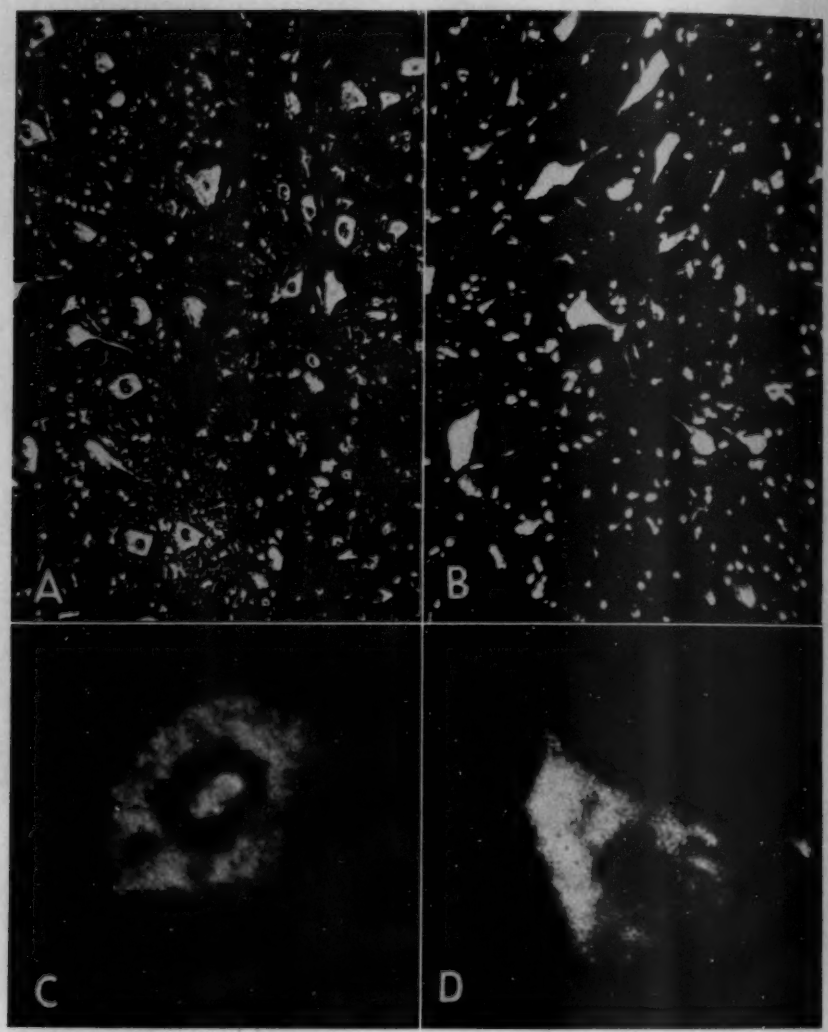

Fig. 3.—*A* gives a low power view (× 162) of a micro-incinerated section of the ventral horn of a normal guinea-pig. The outlines of the cells are regular, and the nuclei are well shown. The nucleoli appear as small white dots. *B* presents the low power view (× 162) of a micro-incinerated section of the ventral horn of guinea-pig 8, which received mussel poison over a period of three days. At this magnification there appears to be little difference between figures 3 *B* and 3 *A*. The glia cells are slightly larger in this section. *C* shows the distribution of ash in a cell of the ventral horn of a normal animal; × 810. The cell is of the same field as shown in *A*. *D* illustrates the distribution of ash in a cell of the ventral horn of a poisoned animal (guinea-pig 8); × 810. Note the unequal distribution of ash throughout the cytoplasm.

the cells of chronically poisoned animals revealed slight thickening, accompanied by diminution in their ability to stain near the center of the cell.

Incinerated sections of tissues from the central nervous systems of acutely poisoned animals differed in no way from sections of the same tissues of normal animals, similarly treated. Nerve tissue from chronically poisoned animals revealed certain changes. These were mild proliferation of the glial elements in sections of the spinal cord and medulla and changes in the ash content of the cells of the ventral horn of the spinal cord and the large ganglion cells of the medulla. In figure 3, *A* and *C* show the appearance of the ash in normal nerve cells under low and high power magnification, respectively. A comparison of *A* and *C* with *B* and *D* serves to illustrate the differences in appearance of the ash in the cells of the ventral horn. There is obviously an unequal distribution of ash in the cell in *D* as compared with that in *C*. The cell outlines in the poisoned animal are less regular than those in the control (fig. 3 *A* and *C*).

COMMENT

The action of mussel poison on the central nervous system is similar to that in other acute intoxications. Certain poisons and toxins, the action of which results in immediate death, are known to provoke no histologic evidence of damage to nerve tissues. Repeated small doses of these poisons, sufficient to produce a chronic condition of poisoning, usually alter cytologic constituents of certain nerve cells. In mussel poisoning, it is the large nerve cells of the spinal cord and medulla which are attacked.

The moderate chromatolysis, the accompanying changes in the neurofibrils and the disturbance in the distribution of ash in these cells are the results of the poisoning. In the ganglion cell of the spinal cord the appearance of the Golgi apparatus is of special significance. The distribution of the Nissl substance is one of the important criteria for judging damage to these cells. Its appearance in the normal nerve cell has been described by Bensley and Gersh,[8] using the freezing-drying method. While it has been shown to be influenced by a variety of agents, such as alcohol or fatigue, suitable fixation is important if any moderate changes demonstrated in it are to be ascribed to a particular source. The examination of adequate control material similarly treated is necessary. Patton[9] found in the incinerated ventral horn cells of monkeys with poliomyelitis a drastic depletion of the ash content, which he believed might be correlated with the extensive chromatolysis of the same cells. Although reduction in the amount of Nissl substance is not a particularly prominent feature of cells containing Negri bodies of rabies, the greater part of the ash is contained within the incinerated inclusion bodies (Covell and Danks[10]). Undoubtedly some of the inorganic ash is derived from sources other than Nissl substance.

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9. Patton, W. E.: Proc. Soc. Exper. Biol. & Med. **31**:195, 1933.
10. Covell, W. P., and Danks, W. B. C.: Am. J. Path. **8**:557, 1932.

The resistance of the mitochondria to mussel poison is also in accordance with the observations for other poisons and toxins. Recently Horning [11] found no alterations, either qualitative or quantitative, in the appearance of the mitochrondria of nerve cells of animals chronically poisoned with morphine. He did, however, observe in certain ganglion cells of the spinal cord a tendency to dispersion of the Golgi apparatus. The results obtained in chronically mussel-poisoned animals differ from these, since the Golgi apparatus appeared clumped in one part of the cytoplasm and dispersed in another part. Battistessa [12] described a reduction in size and a condensation of the apparatus after strychnine and after lead poisoning in animals.

That the changes in the nerve cells of the medulla and spinal cord are due to direct central action of the poison seems probable. Experimental and clinical observations are in agreement. Evidence for an initial reaction to the poison elsewhere than in the nerve cells is lacking. There is no perivascular infiltration such as is characteristic of mushroom poisoning (Marcovitz and Alpers [13]) or destruction of endothelium of blood vessels as occurs in botulism (Meyer [14]). The only striking evidence within the organs is the altered mitochondria of the convoluted tubules of the kidney. The gradual elimination of the poison by the kidneys may be responsible for this effect.

Perhaps the peripheral nervous system and in particular the end-organs will reveal the earlier changes. Judging from the clinical paresthesias which early accompany the poisoning, the peripheral nervous system may be involved.

SUMMARY

Acutely poisoned animals reveal no alterations in the nerve cells of the central nervous system.

The large nerve cells of the ventral horn of the spinal cord and the ganglion cells of the medulla of the chronically poisoned animal show alterations in certain of the cytologic constituents. The mitochondria are normal in appearance in the nerve cells but reveal definite damage in the convoluted tubules of the kidney. The Golgi apparatus of the small and of the medium-sized ganglion cells of the spinal cord is condensed in one part and slightly dispersed in another part of the same cell.

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12. Battistessa, in Cowdry, E. V.: General Cytology, Chicago, University of Chicago Press, 1924, p. 348.

13. Marcovitz, E., and Alpers, B. J.: Arch. Neurol. & Psychiat. **33**:53, 1935.

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WAVE MECHANICS OF PROTOPLASMIC ACTION

XII. EXPERIMENTAL ACUTE TRAUMATIC SYRINGOMYELIA

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The physical state of the axon (axis-cylinder) of a nerve fiber is unknown. There are four theories as follows: (1) the liquidity or fluidity theory (von Kuppfer,[1] Neumann,[2] Jenkins and Carlson,[3] Carlson,[4] Parker,[5] Carey [6]); (2) the semifluidity theory (Ramón y Cajal [7]); (3) the theory of a rodlike solid (von Kölliker [8]), and (4) the theory of a soft gelatinous rod (de Renyi [9]). The remarkable but unsubstantiated statement of Ambrose Paré [10] follows: "The nerves are ways and instruments of the animal spirit. Their figure is round, and long, like a Conduit-Pipe to carry water in."

Meek and Leaper [11] devised a method of fluid transmission of external pressure applied to nerve and muscle. With a constant hydraulic pressure of 40 pounds (18 Kg.) applied around the exterior of the frog's sciatic nerve, it took seven and a half minutes to block the nerve impulse. When applied around the fresh sartorius muscle, it took four and a half minutes to inhibit motion. Nerve and muscle offer great resistances to lateral compression. Pressure as high as 80 and 90 pounds (36 and 41 Kg.) did not block at once the nerve impulse conducted through the sciatic nerve.

The purpose of this paper is the presentation of histologic evidence, portrayed by photomicrographs, of (1) the immediate production of

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5. Parker, G. H.: Quart. Rev. Biol. **4**:155, 1929.

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7. Ramón y Cajal, S.: Textura del sistema neviroso del hombre y de los vertebrados, Madrid, N. Moya, 1899, vol. 1, p. 151.

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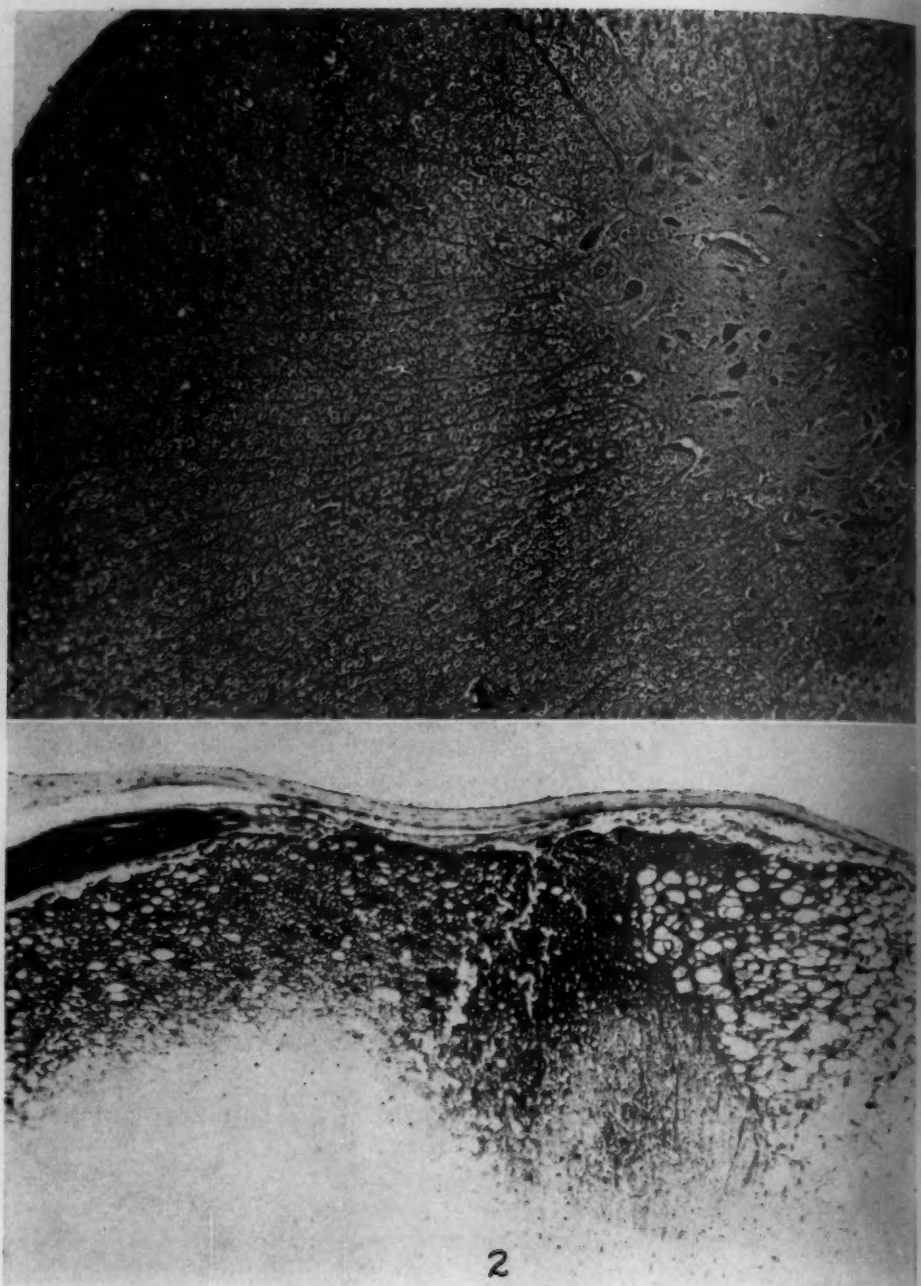
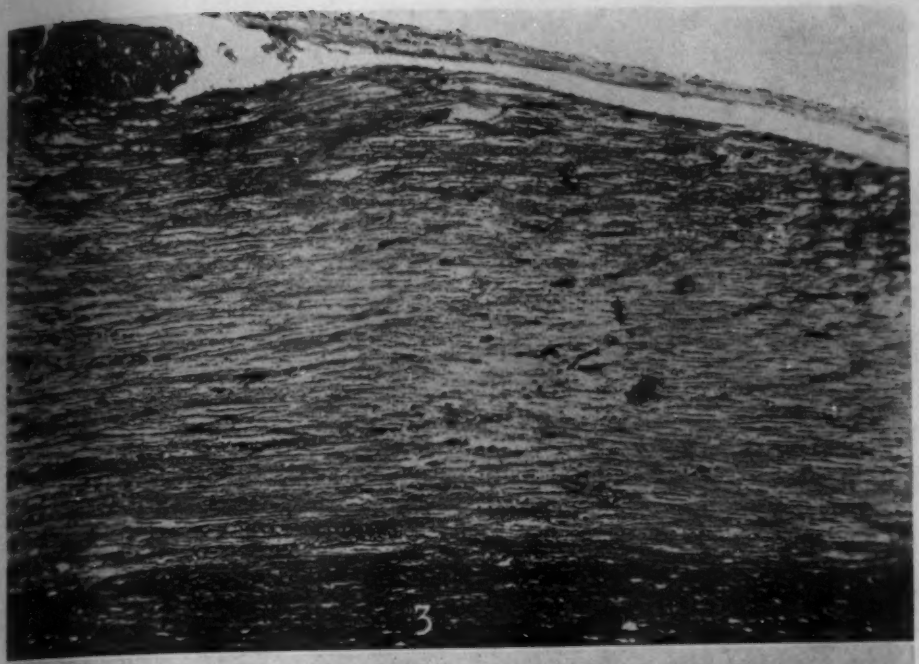
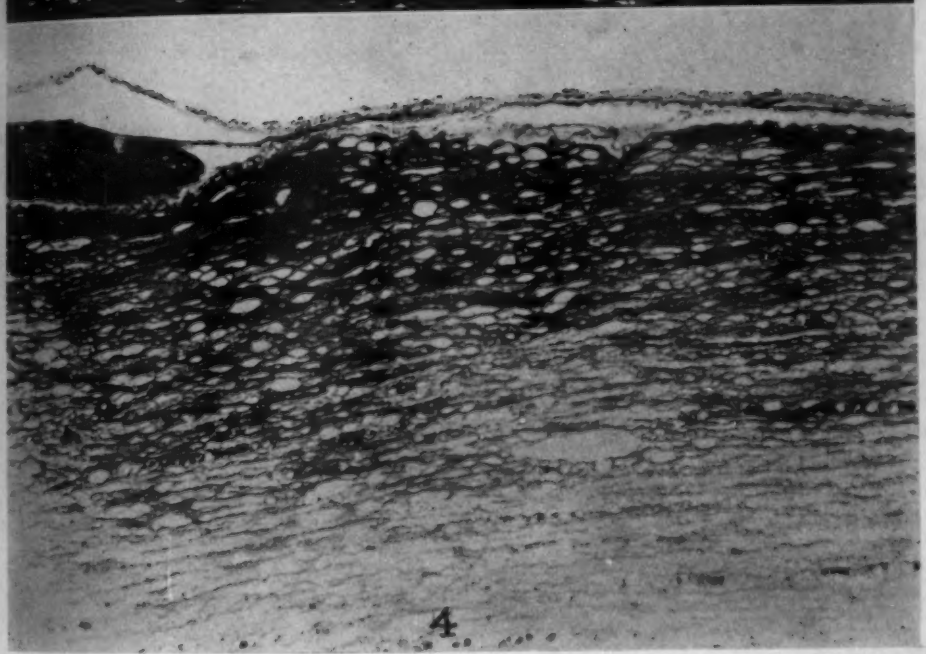


EXPLANATION OF FIGURES 1 AND 2

Photomicrographs (untouched) of transverse sections, 10 microns thick, of the spinal cord at the level of the sixth dorsal segment of the rabbit (Lepus domesticus); × 125; formaldehyde-thionine-dioxane technic. Figure 1 shows a cross-section of the ventral horn and funiculus of the normal sixth thoracic segment of the spinal cord of a rabbit killed in two minutes with ether. Figure 2 shows a cross-section of the dorsal horn and funiculus of the sixth thoracic segment of the spinal cord of a rabbit killed by four hammer blows on the occiput that crushed the medulla oblongata. There are definite overexpansion of many of the axons of the fiber tracts and abnormal compression of related axons. This is histologic evidence of the transmission of abnormal fluid pressure in a cephalocaudal direction, away from the region of high liquid pressure in the axis-cylinders within the medulla oblongata. The central canal is not visible, but it was abnormally dilated throughout its entire extent below the region of experimental trauma.



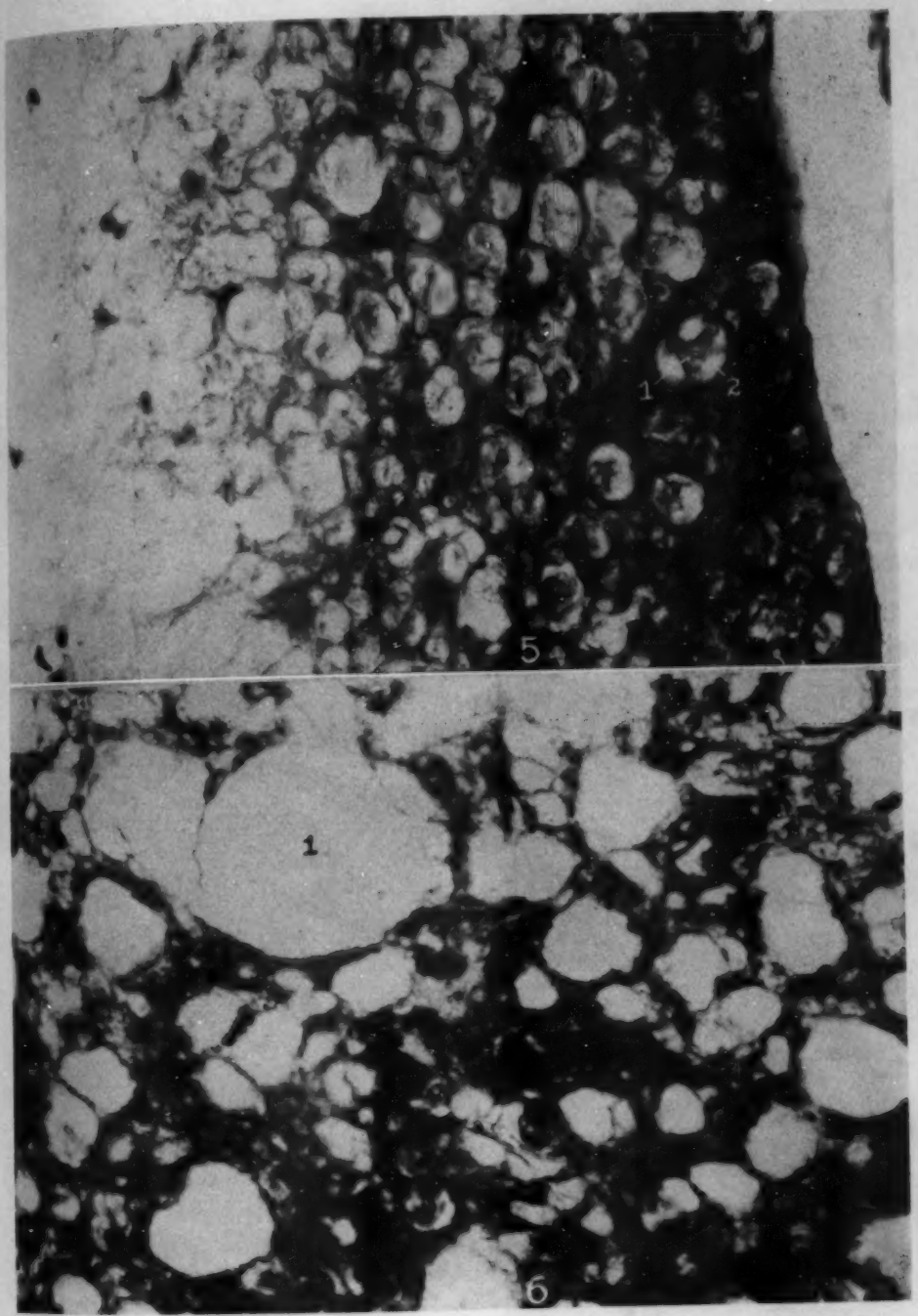


EXPLANATION OF FIGURES 3 AND 4

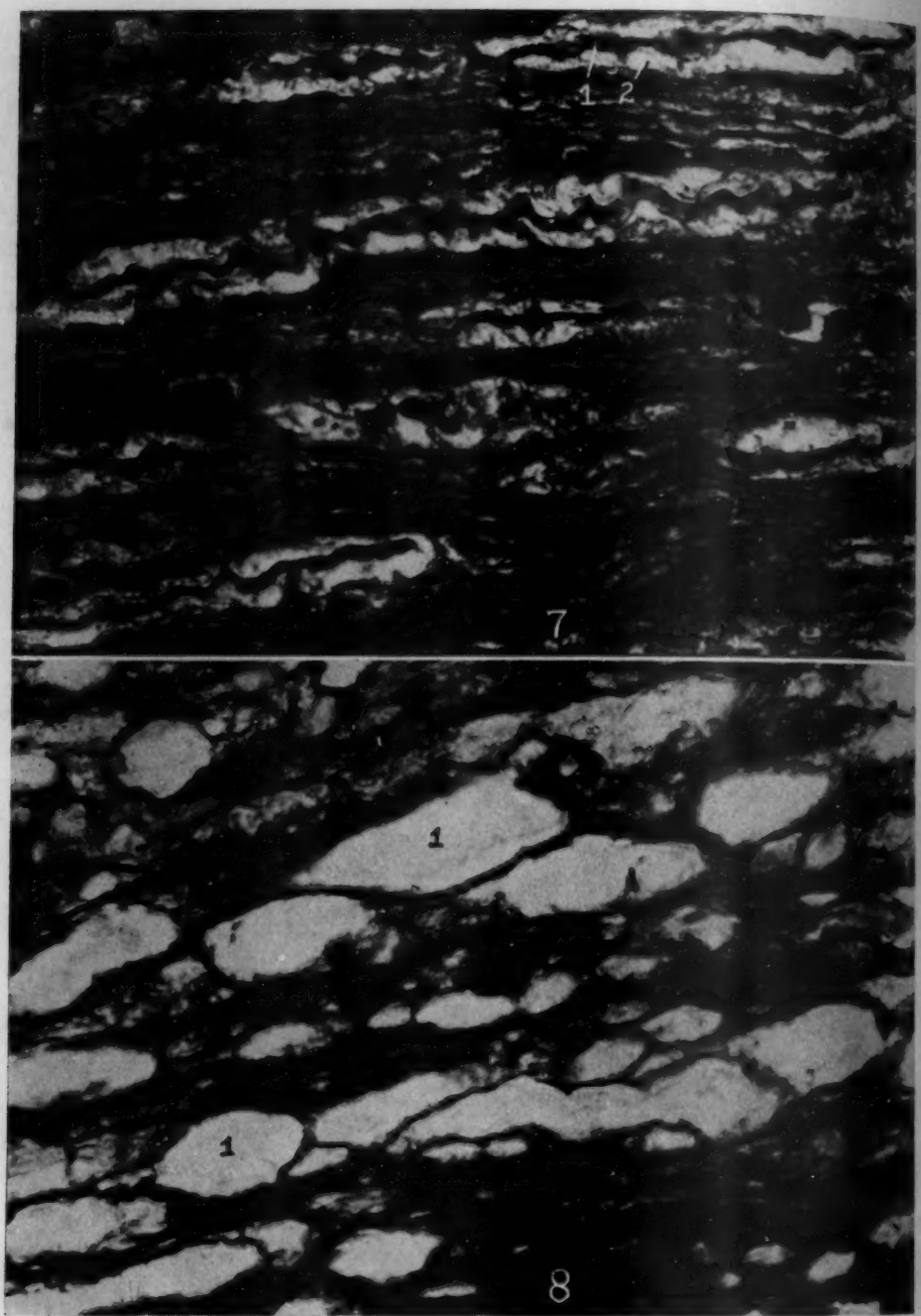
Photomicrographs (untouched) of longitudinal sections, 10 microns thick, of spinal cord at the level of the sixth dorsal segment of the rabbit (Lepus domesticus); × 125; formaldehyde-thionine-dioxane technic. Figure 3 shows a longitudinal coronal section of the lateral funiculus of a normal spinal cord. Figure 4 shows a longitudinal coronal section of the lateral funiculus of the cord from a rabbit killed by hammer blows over the occiput that suddenly crushed the medulla oblongata. The abnormal dilatations of the axons of the motor and sensory fiber tracts are obvious. A sudden high gradient of liquid pressure from the cephalic toward the caudal part of the spinal cord is created by the hammer blows on the occiput. This results in a sudden severe and acute traumatic syringomyelia not alone of the central canal but likewise of certain axons of the fiber tracts of the spinal cord.

EXPLANATION OF FIGURES 5 AND 6

Photomicrographs (untouched) of cross-sections, 10 microns thick, of the lateral funiculus of the spinal cord at the level of the sixth dorsal segment of the rabbit (Lepus domesticus); × 650; formaldehyde-thionine-dioxane technic. Figure 5 shows a cross-section of part of the lateral funiculus of the normal spinal cord from a rabbit killed with ether. Figure 6 shows a cross-section of a part of the cord comparable to that in figure 5, from a rabbit killed by hammer blows over the lower part of the occiput that crushed the medulla oblongata. In these figures *1* indicates an axon; *2*, myelin. The abnormal dilatations and confluences of many axons of the fiber tracts and compression of others are evident. There is a gradient of axonic fluid pressure directed away from the traumatized high pressure area in the medulla and toward the relatively low pressure region at the caudal end of the spinal cord. At vulnerable places certain axons, favored mechanically by the conduction of the high liquid pressure, balloon out in a lateral direction. Where an attenuated intermediate wall is produced between neighboring dilatations there occurs a break in the partition. By this means dilatations of the axons, abnormal in size, are produced.



Figures 5 and 6



EXPLANATION OF FIGURES 7 AND 8

Photomicrographs (untouched) of coronal sections, 10 microns thick, of the dorsal funiculus of the spinal cord at the level of the sixth dorsal segment of the rabbit (Lepus domesticus); × 720; formaldehyde-thionine-dioxane technic. Figure 7 shows a coronal section of the normal spinal cord from a rabbit killed with ether. Figure 8 shows a coronal section of a part of the cord comparable to that in figure 7, from a rabbit killed by hammer blows over the lower part of the occiput that crushed the medulla oblongata. In these figures *1* indicates an axon; *2*, myelin. The abnormal dilatations of certain axons of the fiber tracts and compression of others are definite.

cavities in the lower part of the spinal cord following lethal pressure blows over the medulla oblongata and upper cervical segment of the cord and (2) the outflow of fluid from the cut end of the sciatic nerve stimulated by the faradic current. The experiments demonstrate the liquid nature of the axis-cylinders and the conduction of pulses of pressure through the nerve.

METHOD

Eight rabbits are used in the first experimental series. The two used as normal controls are quickly killed with ether. The other six rabbits are suspended vertically by their hindlegs. Each rabbit is killed with four repeated blows of a hammer over the base of the occiput and upper segment of the cervical region. After laminectomy the spinal cords in both the etherized controls and the experimentally traumatized rabbits are fixed in situ by the formaldehyde-thionine-dioxane method for ninety-six hours. The histologic technic used in the preparation of the cord for observation was outlined in paper XI of this series.[12] The cord of one of the etherized controls is cut in cross-section; the other, in longitudinal section. There are no abnormal cavitations of the spinal cord in the two controls. In the experimentally traumatized rabbits, three of the spinal cords are cut in cross and three in longitudinal sections.

A cardboard is placed beneath the intact median nerve of the rabbit. The nerve is washed free from blood and tissue fluid with pure 0.85 per cent sodium chloride. This is done to remove calcium and organic coagulating agents surrounding the nerve. The nerve is cut transversely and then stimulated 4 cm. away from the incision with the faradic current for one minute. After the lapse of five minutes the nerve is prepared for microscopic observation according to the formaldehyde-thionine-dioxane method.[12]

EXPERIMENTAL RESULTS

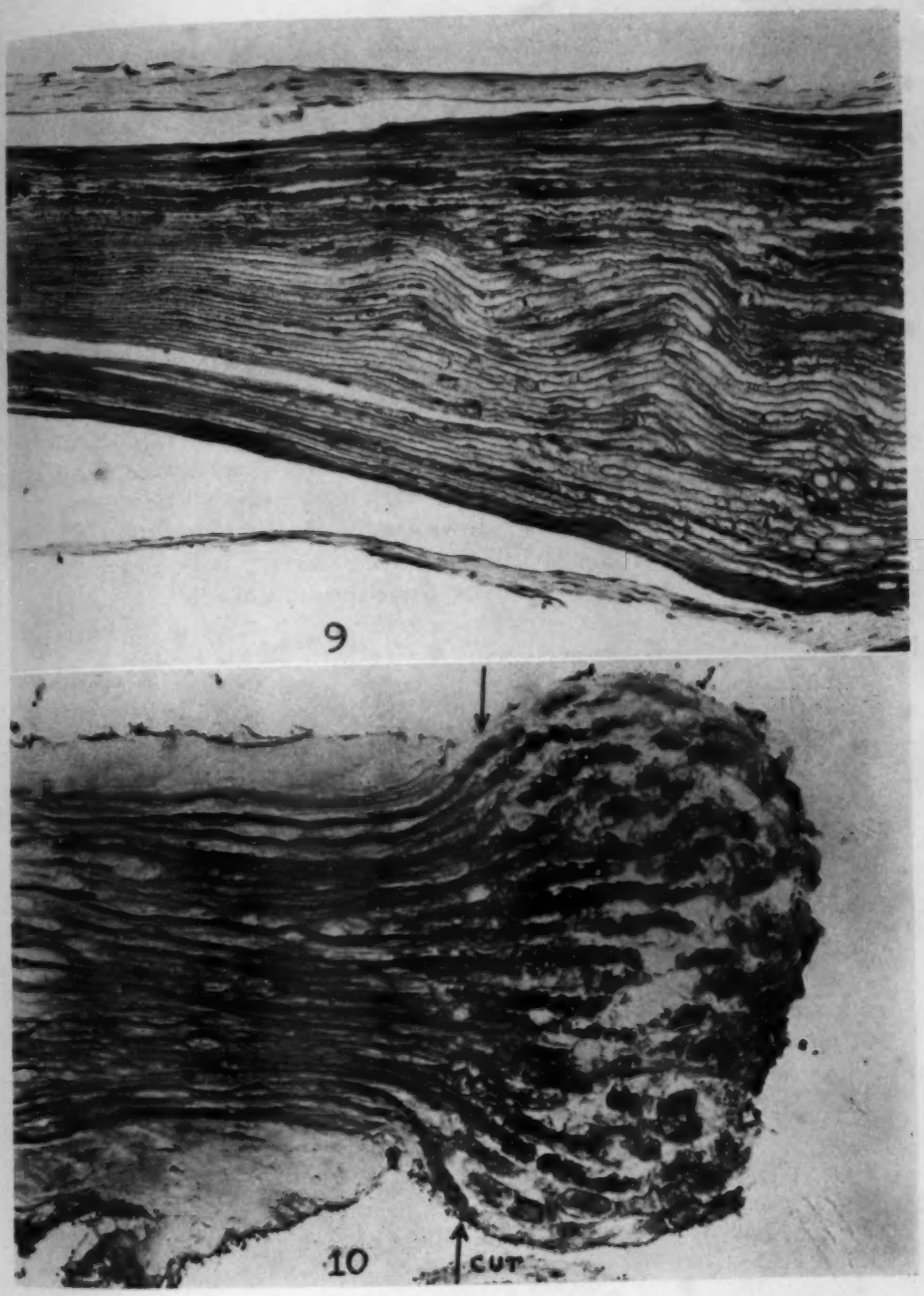
In the midthoracic region of the spinal cord of the rabbit killed by crushing blows over the base of the occiput and upper cervical regions, there are immediately transmitted waves of pressure through the liquid axis-cylinders of the tracts. These waves radiate away from the traumatized region of high pressure toward a zone of low pressure. There are abnormal dilatations of the axis-cylinders of the tracts in the dorsal funiculi of the spinal cord (fig. 2). These structures may be compared with those of the cross-section of a normal cord at the same level and magnification (fig. 1). These cystic-like cavities are suddenly produced by the radiation from the region of high pressure at from 6 to 8 inches (approximately 15 to 20 cm.) above. Some of the axis-cylinders are abnormally expanded; others closely related are compressed (fig. 2). The same comparison of the normal with the abnormally dilated axis-cylinders is made in longitudinal sections (figs. 3 and 4). The cavities of the axis-cylinders are found throughout the sensory and motor tracts in the lateral and ventral funiculi.

12. Carey, Eben J.: Arch. Path. **24**:325, 1937.

EXPLANATION OF FIGURES 9 AND 10

Fig. 9.—Photomicrograph (untouched) of the median nerve trunk of the rabbit (Lepus domesticus); × 125; formaldehyde-thionine-dioxane technic. At the left of the section the nerve fibers are narrow and constricted; toward the right the axons of the fibers are dilated. In this dilated region the spectrum of short dilatations separated by constrictions of the axon is found. This is the histologic expression of part of the nerve impulse conducted synchronously through many neighboring fibers and coagulated in place by the fixative. The lateral expansions of the nerve fibers are evidence of the liquid content of the axons.

Fig. 10.—Photomicrograph (untouched) of the median nerve trunk of the rabbit (Lepus domesticus); × 175; formaldehyde-thionine-dioxane technic. The median nerve trunk was cut transversely after it was raised, placed on cardboard paper and washed with 0.85 per cent sodium chloride solution. This was done to reduce as much as possible the coagulating influence of thrombin and calcium of the blood and tissue juice. After the nerve was sectioned it was stimulated by the faradic current and then placed at once in the fixative and staining solution of formaldehyde-thionine-dioxane. The expansion toward the right of the sectioned median nerve trunk is composed of serial coagulated droplets of fluid, expressed from the axons by the intermittent pressure action of the faradic current. This is direct histologic evidence of the liquid nature of the axons of nerve fibers. There is direct continuity of the nonsegmental narrowed axons on the left with the segmental and discontinuous axonic droplets on the right. Between the axonic liquid droplets there are those composed of the liquid lipoidal myelin which have been dissolved by the dioxane. Arrows are in the direction of the transverse section of the median nerve.



Figures 9 and 10

By a close inspection of both cross and longitudinal sections under high magnifications, it is clearly perceived that some of the greatly enlarged cavities of the traumatized cord are formed by the confluence of two or more swollen neighboring axis-cylinders (figs. 6 and 8). This fusion is the result of the acute collapse of the attenuated walls between closely related and suddenly dilated axis-cylinders. The sudden distention of the axis-cylinders by the augmented internal pressure generated by trauma may occur in any part of the cord at some distance from the initial site of injury. The cross and longitundinal sections of the normal spinal cord (figs. 5 and 7) allow comparisons of the degree of the histologic changes in the cavitations and acute cystic formations caused by trauma.

When the median nerve is cut transversely and stimulated intermittently with the faradic current, the liquid axis-cylinders flow out from the cut end in droplets (fig. 10). There is an increase in the diameter of an active part of a nerve trunk in comparison with the relatively inactive stretched and narrow part of the same nerve (fig. 9). This change in diameter is found in the nerve which is isolated under abnormal stimulation with the induced current and while the coagulative changes of fixation are quickly taking place. The zone of transition between the relatively active and inactive parts of the isolated nerve trunk is preserved (fig. 9).

SUMMARY

The physical state of the axis-cylinders in the central and peripheral nervous systems is that of a colloidal liquid. The experimental production of acute traumatic syringomyelia with multiple cavitations of the axis-cylinders of the spinal cord is dependent on the liquid transmission of pressure pulses from the high to the low pressure area by the sudden crushing blow on the central nervous system. The excessive lateral expansions with "blow outs" of the axis-cylinders by the pressure of mechanical trauma or strychnine stimulation [12] are the result of the liquid transmission of compression. When a nerve is sectioned and stimulated by the faradic current, liquid droplets of the axis-cylinders are expressed from the cut end by the compression pulses of the electric current. The transmission of the nerve impulse tends to produce multiple lateral expansions of the liquid axis-cylinders which conduct the pressure pulses.

By multiple reflections of the abnormal pressure waves amplification occurs and abnormal dilatations or fusions of cavities of many neighboring axis-cylinders result at vulnerable places, where external resistance is diminished and internal pressure great. These experiments likewise suggest the possibility that the acute traumatic syringomyelia produced

in the spinal cord of the rabbit by a sudden blow may be one etiologic factor in certain chronic forms of syringomyelia. It is possible that injuries of the back and trauma of the peripheral nerves may be reflected at a distance from the initial site of injury by the transmission of augmented pressure through the liquid axis-cylinders. Not only may the secondary site of trauma in the spinal cord be remote in space from the original site of injury, but its detection by secondary degenerative and reparative gliomatous changes becomes remote in time from the incidence of the original lesion.

AVITAMINOSIS

XIX. NERVE DEGENERATION IN ALBINO RATS AS STUDIED BY THE FREEZING-DRYING METHOD AND POLARIZED LIGHT WITH DEFICIENCY OF VITAMIN A OR OF VITAMIN B

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The literature on nerve degeneration in avitaminosis is extensive and conflicting. Therefore, in reviewing the results of previous investigations in this field, we shall review only such studies as are pertinent to our own investigations.

The early literature on vitamin B was reviewed by Zimmerman and Burack,[1] who found extensive demyelinization in the sciatic, median, ulnar and vagus nerves and in the brachial plexuses of dogs on diets deficient in the vitamin B complex. The extent of the histologic changes in the peripheral nerves varied in direct proportion to the severity and duration of the polyneuritic symptoms. Similar changes, but of a less degree, were observed in starved dogs which had been provided with a sufficiency of the vitamin B complex.

Moore, Plymate and Andrew [2] made a study of nerve changes in 471 rats chosen from stock which had been fed eighteen different synthetic diets deficient in the vitamin B complex. The following peripheral nerves were examined: the sciatic nerves, the phrenic nerves, the vagi and the brachial plexuses. The larger branches were studied, no examination being made of the nerve endings. The nerves were fixed and stained with osmic acid. These investigators stated that no more wallerian degeneration was seen in the nerve preparations of the avitaminotic rats than in those of the controls. The fact that a number of the control animals showed demyelinization of peripheral nerves is evidence that the technic of fixation with osmic acid and staining lacks reliability from the standpoint of circumventing postmortem changes and does not present the picture of the living neuron.

Research Paper no. 455, Journal Series, University of Arkansas.

1. Zimmerman, H. M., and Burack, E.: Arch. Path. **13**:207, 1932.

2. Moore, C. U.; Plymate, H. B., and Andrew, B. J.: Am. J. Physiol. **102**: 598, 1932.

Grinker and Kandel [3] studied rats on diets deficient, respectively, in the vitamin B complex, vitamin A and vitamin B_1 but found no changes in the central nervous system. In discussing Grinker and Kandel's paper, Dr. H. M. Zimmerman stated that he had found symptoms pointing to involvement of the central nervous system in dogs but not in rats deprived of the water-soluble B vitamins.

Prickett [4] was the first to study the peripheral and central nervous system in a group of rats showing uncomplicated dietary deficiency of vitamin B_1. The peripheral nervous system, including the sciatic nerves and brachial plexuses, was studied, osmic acid and sudan III being used as stains. From his data Prickett concluded that the lesions were central rather than peripheral. In approximately 75 per cent of the deficient animals he observed hemorrhages which involved the nucleus of Deiters, the chief vestibular nucleus, the nucleus of Bechterew and the nucleus solitarius. Prickett's findings in the central nervous system are not in accord with those of Grinker and Kandel [3] or those of Zimmerman in studies of the rat.

Mellanby [5] was the first to report nerve lesions in animals (puppies) on a diet deficient in fat-soluble vitamins.

Hughes and co-workers [6] reported nerve lesions due to vitamin A deficiency in pigs, chickens and cows.

Aberle [7] found extensive degeneration of the medullary sheaths of the brachial plexus and sciatic nerves in rats on diets deficient in vitamin A, which accounts for the muscular incoordination frequently observed in animals with this avitaminosis.

Mellanby [8] reported degeneration of peripheral nerves due to vitamin A deficiency in dogs. The peripheral degeneration, he stated, is responsible for the excessive incoordination and abnormal movements of the head so often encountered. Similar degeneration of fibers was found in the cochlear nerve. Degenerative changes were found in the posterior roots of the spinal nerves while the anterior roots of the same animal escaped degenerative changes. He therefore concluded that it is the afferent nerves which are especially liable to degenerate in A avitaminosis. The finding of degeneration in afferent fibers of

3. Grinker, R. A., and Kandel, E.: Arch. Neurol. & Psychiat. **30**:1287, 1933.

4. Prickett, C. O.: Am. J. Physiol. **107**:459, 1934.

5. Mellanby, E.: J. Physiol. **61**:xxiv, 1926.

6. Hughes, J. S.; Aubel, C. E., and Lienhardt, H. F.: The Importance of Vitamin A and Vitamin C in the Ration of Swine, Concerning Especially Their Effect on Growth and Reproduction, Technical Bulletin 23, Kansas Agricultural Station, 1928, p. 1.

7. Aberle, S. B. D.: J. Nutrition **17**:445, 1934.

8. Mellanby, E.: Nutrition and Disease: An Interpretation of Clinical and Experimental Work, London, Oliver & Boyd, 1934, pp. 109-111.

the trigeminal nerves which are sensory to the conjunctiva and cornea led Mellanby to postulate the hypothesis that xerophthalmia, beginning with first signs of keratomalacia, is caused by degeneration of afferent nerves innervating various surfaces of the eye.

None of the investigators cited controlled the plane of nutrition of their animals; therefore, the amount of food ingested may have been a complicating factor. Furthermore, the Marchi method of staining and the method of fixation employed by the majority of the workers frequently lead to artefacts which may, to a great extent, account for the conflicting literature. Wolbach,[9] in his discussion of the pathologic changes resulting from vitamin deficiencies, refers to it as "the capricious Marchi technic." Swank and Davenport [10] recently reported artefacts by this procedure, while Zimmerman and Burack [1] called attention to artefacts produced by the Spielmeyer method. In order to prevent uncertainties introduced by such procedures, Sutton, Setterfield and Krauss [11] applied the polarized light technic to the study of myelinated nerves in vitamin A deficiency. This technic, however, as employed by Sutton and associates requires previous fixation of tissues in solution of formaldehyde, a procedure which may introduce artefacts. We have applied the technic of Sutton and co-workers in studies of deficiency of the vitamin B complex, deficiency of vitamin B_1 and deficiency of vitamin A in albino rats and have checked the procedure of fixation in solution of formaldehyde by eliminating all fixatives as well as all staining operations. Throughout this investigation we carried out the experiments in pairs, using litter mates of the same sex and restricting the controls to the same plane of nutrition as the avitaminotic animals. A comparison of the rations is given in table 1.

The animals of the groups deficient in the vitamin B complex received ration 1751 supplemented with 4 drops of cod liver oil daily. Their controls received ration 1845 and the same number of drops of cod liver oil. The polyneuritic group, animals deficient in vitamin B_1, received ration 3148-X supplemented with 4 drops of cod liver oil daily, and the controls, the same ration and cod liver oil allowance supplemented daily with 10 mg. of vitamin B_1 crystals. The rats deficient in vitamin A were given ration 1034-A irradiated for thirty minutes (with ultraviolet rays from a uviarc lamp), which insured an adequate intake of vitamin D. The controls received the same ration and a daily supplement of 10 drops of cod liver oil.

9. Wolbach, S. B.: J. A. M. A. **108**:7, 1937.

10. Swank, R. L., and Davenport, H. A.: Stain Technol. **10**:45, 1935.

11. Sutton, T. S.; Setterfield, H. E., and Krauss, W. E.: Nerve Degeneration Associated with Avitaminosis A in the White Rat, Bulletin 545, Ohio Agricultural Experiment Station, December 1934.

MATERIAL AND METHODS

Lee[12] stated that true fats are isotropic to polarized light, whereas cholesterol, cholesterol esters, phosphatides, cerebrosides and cholesterol-fatty acid mixtures are anisotropic. According to Mathews,[13] the normal myelin sheath consists of phosphatides, cerebrosides and sulfatids and is almost entirely free from true fats (triglycerides).

Isotropic materials when observed in polarized light and between crossed Nicol prisms appear dark. Triglycerides are isotropic and when present in the myelin sheath are seen as dark bodies in striking contrast to the myelin, which is birefringent.

Small pieces of spinal cord and of trigeminal, sciatic and optic nerves from nine rats deficient in vitamin B_1 and three rats deficient in the vitamin B complex

TABLE 1.—*Composition of Rations*

	Ration 1751	Ration 1845	Ration 1034-A	Ration 3148-X
Casein	20*	20†	20§	18#
Agar-agar	2			
Salts 185	4	4	4	4
Butter fat (filtered)	10	10		
Dextrin	64	56	66	
Dehydrated brewers' yeast		10	10	
Butter fat (unfiltered)				9
Corn starch				54
Fresh bakers' yeast¶				9
Autoclaved beef‡				6
Cod liver oil				2
	100	100	100	100

* The casein was washed with acidulated water for ten days and then extracted several times in 25 per cent ethyl alcohol at room temperature.
† The casein was washed with acidulated water for ten days.
§ The casein was extracted three times with hot ethyl alcohol.
# A commercial casein was used.
¶ This yeast was autoclaved for five hours at 15 pounds' (6.8 Kg.) pressure at the natural p_H of the yeast.
‡ This was autoclaved for six hours at 15 pounds' pressure at the natural p_H of the beef.

and their litter mate controls, which were on the same plane of nutrition, were fixed in 95 per cent alcohol in liquid air. They were then dehydrated in vacuo at —32 C. by the Altman-Gersh freezing-drying method as modified by Scott and Williams.[14] They were then cleared in vacuo by glycerin according to the method of Hoerr,[15] sectioned at 20 microns with the freezing microtome and studied with polarized light between crossed Nicol prisms.

Small pieces of spinal cord and of sciatic, trigeminal and optic nerves from the same groups of animals were fixed in solution of formaldehyde U. S. P. (1:10) for twenty-four hours before sectioning. Frozen sections of these were made for comparison with those fixed in liquid air.

12. Lee, A. B.: Microtomists Vade Mecum, ed. 8, Philadelphia, P. Blakiston's Son & Co., 1921, p. 361.

13. Mathews, A. P.: Physiological Chemistry, ed. 4, Baltimore, William Wood & Company, 1925, p. 606.

14. Scott, G. H., and Williams, P. S.: Anat. Rec. **66**:475, 1936.

15. Hoerr, N. L.: Anat. Rec. **66**:91, 1936.

The remainder of the material, consisting of the same nerves from thirteen rats deficient in vitamin B_1, ten deficient in the vitamin B complex and twenty deficient in vitamin A and from their restricted controls, were fixed in solution of formaldehyde U. S. P. (1:10) previous to examination with polarized light.

Other small pieces of tissue, representing the pons, cerebrum and cerebellum, from the aforementioned rats deficient in vitamin B_1 and vitamin B complex and their restricted controls were fixed in liquid air, dehydrated in vacuo, embedded in paraffin, sectioned at 8 microns and stained with either hematoxylin and eosin or Giemsa's stain.

RESULTS [16]

The nerve material which had been fixed in liquid air was compared with that fixed in solution of formaldehyde U. S. P. (1:10). This comparison demonstrated differences between the two methods of

TABLE 2.—*Nerve Degeneration in Vitamin B_1 Deficiency Associated with Polyneuritis* *

Rat	Days on Deficient Diet	Loss of Weight, per Cent	Microscopic Diagnosis†				Degree of Paralysis
			Sciatic Nerve	Trigeminal Nerve	Spinal Cord	Optic Nerve	
1	44	30	+++	+++	++	0	Marked paralysis, convulsions
2	22	29	++	++	+	0	Partial paralysis
3	60	25	++	+	0	0	Slight paralysis
4	59	23	+++	++	+	0	Marked paralysis
5	20	23	++	++	+	0	Slight paralysis
6	50	21	++	++	++	0	Slight paralysis
7	53	20	++	+++	++	0	Slight paralysis, bleeding of nose
8	40	20	++	+	0	0	Slight paralysis, bleeding of nose
9	42	12	0	0	0	0	Very slight paralysis

* The nerves were frozen in liquid air and examined with polarized light.

† In tables 2 to 6, inclusive, a single plus sign indicates the presence of isotropic material in the myelin sheath and swelling of fibers. Two or three plus signs are used to indicate increasing amounts of isotropic material.

fixation. We were certain, from the aforementioned study, that fixation in solution of formaldehyde previous to observation by polarized light was a dependable procedure.

The results are given in tables 2 to 6, representing the respective groups of vitamin-deficient animals.

Vitamin B_1 (Fig. 1 *B* and *D;* fig. 2 *A, B* and C).—The spinal cord and the sciatic, trigeminal and optic nerves from each of twenty-two polyneuritic and an equal number of restricted control rats were studied The symptoms of the pathologic animals ranged from very slight to marked paralysis with opisthotonos and cart-wheels. These rats were on a diet deficient in vitamin B_1 for periods of from twenty-one days to four months.

The most regular and marked degeneration of myelin was found in the trigeminal and sciatic nerves. The degree of degeneration, in most

16. A preliminary report on the vitamin B studies has been published (Proc. Soc. Exper. Biol. & Med. **35**:583, 1937).

cases, corresponded to the number of days the rats were on the deficient diet and to the progress of the paralysis. The nerve fibers from animals with mild paralysis were swollen, with practically no degeneration of myelin (fig. 2 *B*), while those from animals with marked paralysis (fig. 2 *C*) showed degeneration of myelin and breaking of many of the axis-cylinders.

TABLE 3.—*Nerve Degeneration in Deficiency of the Vitamin B Complex* *

Rat	Days on Deficient Diet	Loss of Weight, per Cent	Microscopic Diagnosis			
			Sciatic Nerve	Trigeminal Nerve	Spinal Cord	Optic Nerve
10	49	28	+	++	+	0
11	54	22	0	+	0	0
12	30	20	+	++	+	0

* The nerves were frozen in liquid air and examined with polarized light.

TABLE 4.—*Nerve Degeneration in Vitamin B_1 Deficiency Associated with Polyneuritis* *

Rat	Days on Deficient Diet	Loss of Weight, per Cent	Microscopic Diagnosis				Degree of Paralysis
			Sciatic Nerve	Trigeminal Nerve	Spinal Cord	Optic Nerve	
13	44	30	+++	++	++	0	Marked paralysis, convulsions
14	22	20	++	++	+	0	Partial paralysis
15	60	25	++	+	0	0	Slight paralysis
16	50	23	+++	++	+	0	Marked paralysis
17	20	23	++	++	+	0	Slight paralysis
18	50	21	++	++	++	0	Slight paralysis
19	34	21	+	++	0	0	Marked paralysis
20	53	20	++	+++	++	0	Slight paralysis, bleeding of nose
21	40	20	++	+	0	0	Slight paralysis
22	42	12	0	0	0	0	Very slight paralysis
23	37	11	+	++	0	0	Marked paralysis, priapism
24	34	20	++	++	+	0	Moderate paralysis
25	21	22	++	++	0	0	Slight paralysis
26	55	16	++	++	0	0	Very slight paralysis
27	43	24	++	+++	0	0	Marked paralysis, opisthotonos; cartwheels
28	25	27	++	++	0	0	Slight paralysis
29	33	23	+++	0	0	0	Marked paralysis
30	49	15	++	++	0	0	Marked paralysis, slight convulsions
31	118	25	++	+++	+	0	Marked paralysis, opisthotonos
32	104	23	++	++	0	0	Convulsions, slight opisthotonos
33	110	19	+++	++	0	0	Convulsions, marked paralysis
34	94	44	++	0	0	0	Convulsions, slight paralysis

* The nerves were fixed in solution of formaldehyde U. S. P. (1:10) and examined with polarized light.

Slight degeneration was observed in the spinal cords of eight pathologic animals. No changes were observed in those of the control group. The amount of degeneration in the spinal cords of the pathologic animals seemed to have no definite relation to the progress of the paralysis. The optic nerves in all pathologic and control rats appeared normal.

The extent of the loss of weight had no definite relationship to the severity of nerve degeneration.

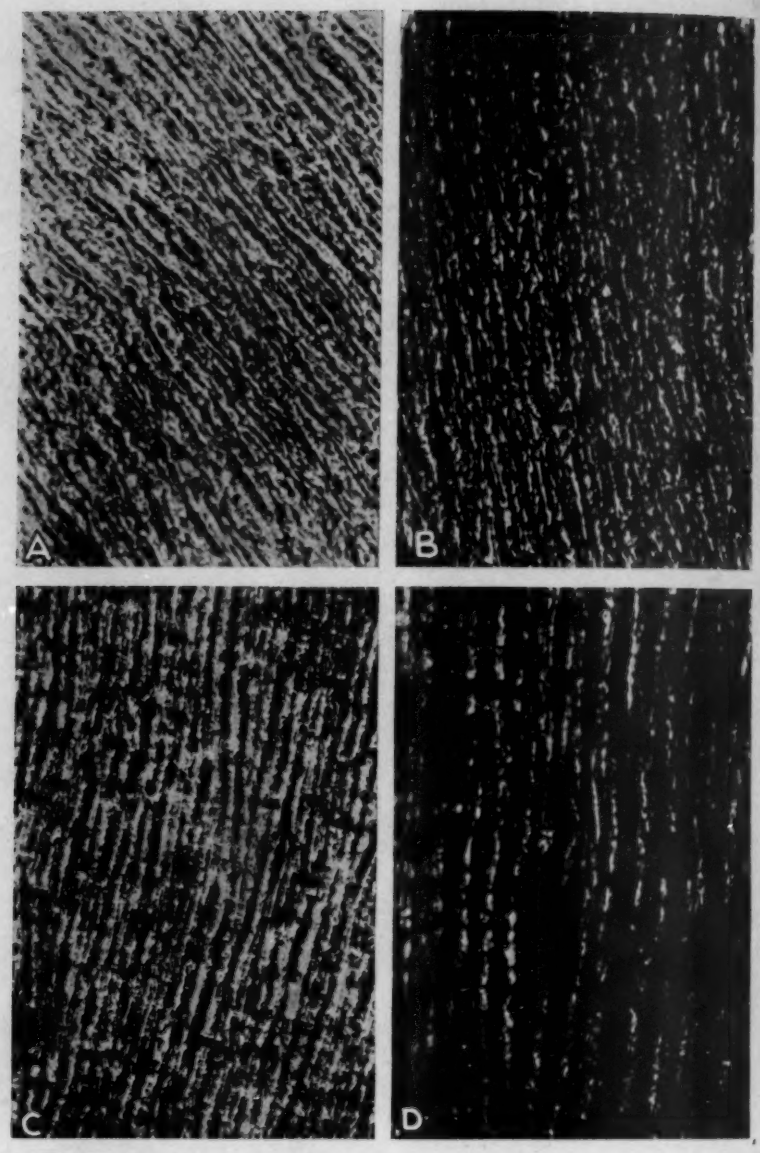

Fig. 1.—*A*, longitudinal section of the trigeminal nerve from restricted control rat 1, fixed in liquid air, dehydrated in vacuo and cleared in vacuo by glycerin. This and the sections subsequently shown were photographed in polarized light and between crossed Nicol prisms at the point of greatest birefringence at a magnification of × 450. *B*, section of the trigeminal nerve from rat 1, which had been on a diet deficient in vitamin B_1 for forty-four days. The tissue was fixed and cleared in the same way as *A*. *C*, section of the trigeminal nerve from restricted control rat 3, fixed in solution of formaldehyde U. S. P. (1:10). *D*, section of the trigeminal nerve from rat 3 fixed in solution of formaldehyde U. S. P. (1:10). Note the swelling of fibers and the degeneration of two or three in the center. This animal was on a diet deficient in vitamin B_1 for sixty days. Compare with *C*.

TABLE 5.—*Nerve Degeneration in Deficiency of the Vitamin B Complex* *

Rat	Days on Deficient Diet	Loss of Weight, per Cent	Microscopic Diagnosis			
			Sciatic Nerve	Trigeminal Nerve	Spinal Cord	Optic Nerve
35	40	33	+	+	0	0
36	49	26	+	++	+	0
37	43	26	0	++	0	0
38	41	25	++	+	0	0
39	54	22	0	+	0	0
40	34	22	++	0	+	0
41	30	20	+	++	+	0
42	35	21	++	++	0	0
43	59	24	+	+	0	0
44	35	21	++	++	0	0
45	100	26	++	++	0	0
46	71	28	++	++	0	0
47	84	26	++	++	0	0

* The nerves were fixed in solution of formaldehyde U. S. P (1:10) and examined with polarized light.

TABLE 6.—*Nerve Degeneration in Vitamin A Deficiency* *

Rat	Days on Deficient Diet	Loss or Gain in Weight, per Cent	Microscopic Diagnosis						Comment
			Sciatic Nerve	Trigeminal Nerve	Ophthalmic Nerve		Spinal Cord	Optic Nerve	
					Right	Left			
48	58	—17	++	+++	++	++	+	0	Pneumonia
49	14	—10	0	0	0	0	0	0	Pneumonia, congestion of right lung, cornification of vaginal epithelium
50	35	+33	0	0	0	0	0	++	Cornification of vaginal epithelium
51	41	+ 6	0	+	0	0	0	0	Slight ophthalmia, cornification of vaginal epithelium
52	49	Still gaining	++	+	0	0	0	+	Slight congestion of lung, cornification of vaginal epithelium
53	45	+ 5	++	++	++	++	0	0	Ophthalmia of right eye, cornification of vaginal epithelium
54	48	—35	++	++	+++	++	0	0	Marked ophthalmia
55	52	—12	++	++	+	+	0	0	Marked ophthalmia, slight congestion of lungs
56	43	— 2	0 (slight swelling)	+	0	0	0	++	Slight ophthalmia of right eye
57	45	—14	++	++	++	++	0	0	Ophthalmia of right eye, pus pocket in tongue
58	51	+ 3	++	+++	0	0	0	+	Marked ophthalmia, pus pocket in tongue, cornification of vaginal epithelium 12 days
59	56	— 1	0	++	++	0	0	++	Marked ophthalmia both eyes, pus pocket in tongue
60	68	— 5	0	++	0	0	0	+	Marked ophthalmia both eyes, cornification of vaginal epithelium 15 days
61	53	—25	++	0	0	0	0	++	Ophthalmia right eye, cornification of vaginal epithelium 14 days
62	64	— 8	++	++	++	++	+	0	Ophthalmia both eyes, cornification of vaginal epithelium 18 days
63	65	— 2	++	0	0	0	0	0	Slight ophthalmia left eye, cornification of vaginal epithelium 11 days
64	53	— 2	0	0	0	0	0	0	
65	49	— 4	+	++	++	0	0	+	Slight ophthalmia
66	60	—23	++	++	++	++	0	++	No ophthalmia; pus pockets in tongue
67	54	—16	+	+	+	+	0	+	No ophthalmia

* The nerves were fixed in solution of formaldehyde U. S. P (1:10) and examined with polarized light.

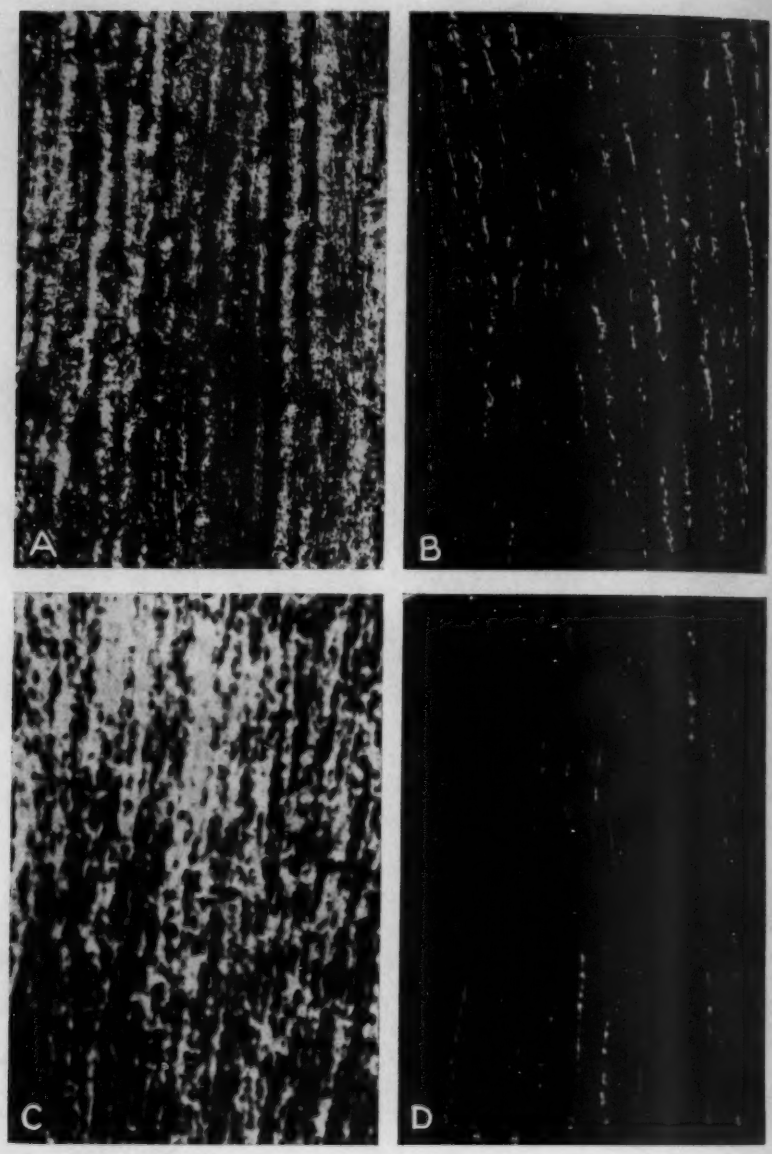

Fig. 2.—*A*, sciatic nerve from rat 17, which was on a diet deficient in vitamin B_1 for twenty days. This and the sections subsequently shown were photographed in polarized light and between crossed Nicol prisms, at a magnification of × 450. *B*, sciatic nerve from rat 24, which had been on a diet deficient in vitamin B_1 for eighty-four days. *C*, sciatic nerve from rat 31. Note the broken axis-cylinders. This animal was on a diet deficient in vitamin B_1 for four months. *D*, sciatic nerve from rat 45, which was on a diet deficient in the vitamin B complex for one hundred days.

Vitamin B Complex (Fig. 3 *A* and *B*).—The spinal cord and the sciatic, trigeminal and optic nerves from sixteen rats deficient in the vitamin B complex and from an equal number of restricted controls were examined. The pathologic rats had been on the deficient diet for from twenty-two to one hundred days.

As in the animals deficient in vitamin B_1, the most pronounced degeneration was observed in the trigeminal and sciatic nerves. In some instances there was only slight swelling of a few fibers. In later stages the degeneration of myelin corresponded to the early changes attributed to deficiency of vitamin B_1. Broken axis-cylinders comparable to those seen in late stages of vitamin B_1 deficiency were not observed. No degeneration was apparent in the controls. Changes in the spinal cord were confined to slight degeneration and swelling of a few fibers. The fibers in the spinal cords of control sections were not swollen.

The optic nerves in both pathologic and control sections appeared normal.

Vitamin A (Fig. 3 *C* and *D*).—The spinal cord and the sciatic, trigeminal, ophthalmic and optic nerves from a group of twenty rats deficient in vitamin A and their litter mate controls were examined. The most marked degenerations were observed in the optic, sciatic, trigeminal and ophthalmic nerves. It can be seen from table 5 that the degeneration of the ophthalmic nerve did not correspond to the onset of ophthalmia and that in some cases there was degeneration of this nerve without external signs of ophthalmia. Some rats showed ophthalmia without nerve degeneration.

Slight swelling of fibers was noted in the spinal cords of two animals; in the remainder of the pathologic rats, as well as in the control animals, there was no degeneration.

Pons, Cerebrum and Cerebellum in Avitaminoses.—After careful study of serial sections from the pons, cerebrum and cerebellum of the polyneuritic rats and the rats deficient in the vitamin B complex, which were fined by the freezing-drying method and stained with the Giemsa or the hematoxylin and eosin stain, no histologic differences between sections from pathologic and those from restricted controls could be detected.

COMMENT

Evidence is gradually being accumulated that the use of polarized light is a dependable procedure for the study of myelin degeneration. Thus, Baldi [17] obtained good results, and later in this country Sutton, Setterfield and Krauss [11] applied the method to nerve degeneration

17. Baldi, F.: Riv. di pat. nerv. **35**:158, 1930.

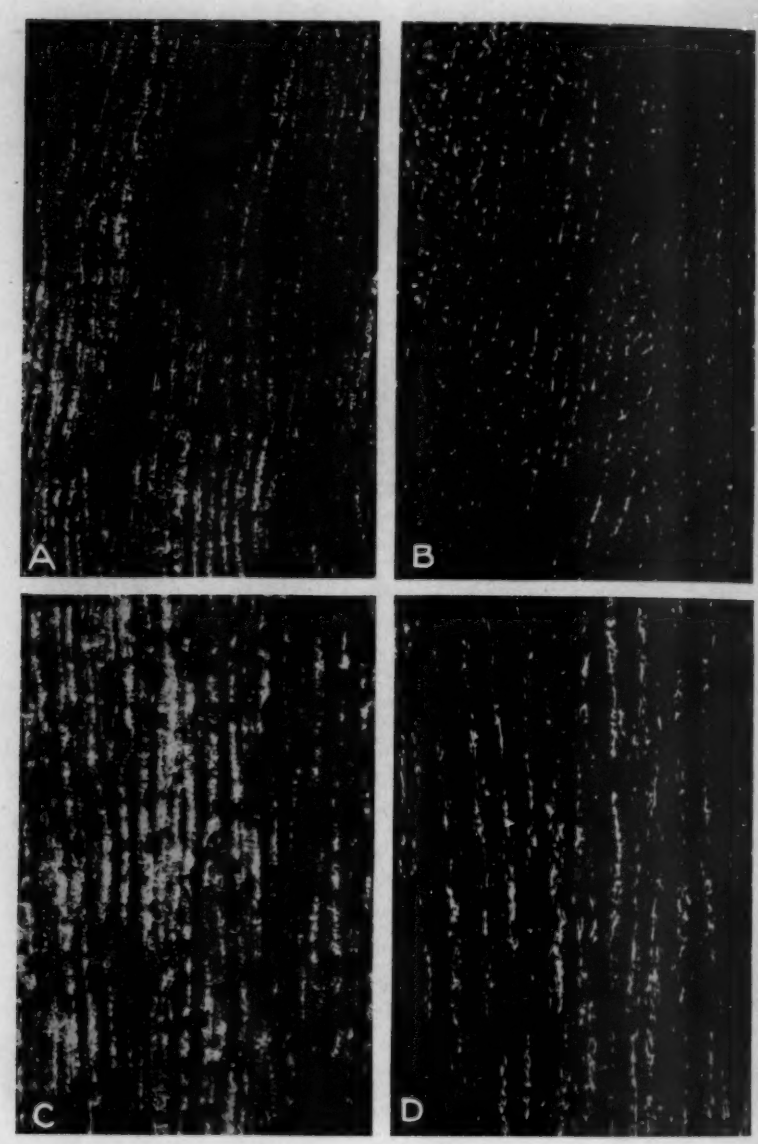

Fig. 3.—*A*, longitudinal section of trigeminal nerve from rat 47, which was on a diet deficient in the vitamin B complex for eighty-four days. This and the sections subsequently shown were photographed in polarized light and between crossed Nicol prisms at a magnification of × 450. *B*, sciatic nerve from rat 41, which was on a diet deficient in vitamin B complex for thirty days. *C*, trigeminal nerve from rat 53, which was on a diet deficient in vitamin A for forty-five days. *D*, trigeminal nerve from rat 58, which was on a diet deficient in vitamin A for fifty-six days.

associated with avitaminosis A in the white rat. Toomey and Weaver [18] recently used it in their study of degeneration of peripheral nerves due to the virus of poliomyelitis. We obtained good results with this method after fixation in either solution of formaldehyde U. S. P. (1:10) or liquid air.

Our results with nerves from animals deficient in vitamin A correspond, in the most part, with those of Sutton, Setterfield and Krauss.[11] We did not find, however, that degeneration of the ophthalmic nerve necessarily appeared at the same time as ophthalmia. Another point in favor of xerophthalmia being of epithelial rather than of nervous origin is the fact that we have found degeneration of the trigeminal nerve in rats deficient in vitamin B_1 and in rats deficient in the vitamin B complex in which no ophthalmia developed. Therefore, we do not attribute this condition to degeneration of the afferent nerves of the eye as does Mellanby.[8]

A possible explanation of tissue changes in ophthalmia lies in the fact that the tissues involved are high in phospholipid. Mori [19] found droplets of fat in the cells of the lacrimal glands, and Monaghan and Schmitt [20] suggested the probability of vitamin A being the regulator of phospholipid metabolism. Wolbach [9] concluded that the change back to the normal epithelium in xerophthalmia is abrupt and that this affords further evidence that the primary consequence of vitamin A deficiency is of epithelial and not of nervous origin. Zimmerman and Cowgill [21] found that when rats which were deficient in vitamin A were treated with carotene they resumed their normal rate of growth and recovered from the xerophthalmia, but that there did not seem to be any concomitant repairs of the nerves in the treated animals.

The results reported in this paper do not correspond to those of Moore and associates.[2] They found no more nerve degeneration in their preparations from avitaminotic rats than in control material. Prickett [4] found changes to be central rather than peripheral. It seems that the changes which they observed might have been due to their methods of fixation and staining.

The findings reported here are in accord with those of Grinker and Kandel [3] except that we found slight swelling of fibers in the spinal cord. They found no changes in the central nervous system.

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18. Toomey, J. A., and Weaver, H. M.: Am. J. Dis. Child. **53**:79, 1937.
19. Mori, S.: Bull. Johns Hopkins Hosp. **33**:357, 1922.
20. Monaghan, B. R., and Schmitt, F. O.: J. Biol. Chem. **96**:387, 1932.
21. Zimmerman, H. M., and Cowgill, G. R.: J. Nutrition **11**:423, 1936.

SUMMARY

Polarized light has been applied satisfactorily in the study of degeneration in myelinated nerves. It has been proved by fixation of nerves in liquid air and comparison of these with nerves fixed in solution of formaldehyde that the latter produces no artefacts.

Spinal cord and sciatic, trigeminal and optic nerves from rats deficient in vitamin B_1 and from other rats deficient in the vitamin B complex were fixed in liquid air or in solution of formaldehyde and studied with polarized light. Also, the same nerves from animals deficient in vitamin A were fixed in solution of formaldehyde and studied by the same method.

In nerves from rats deficient in vitamin B_1 there were varying degrees of myelin degeneration. In the later stages the axis-cylinders were broken. Nerves from animals deficient in vitamin A and from animals deficient in the vitamin B complex showed myelin degeneration, but the axis-cylinders remained intact.

Sections of cerebrum, cerebellum and pons from rats deficient in vitamin B_1 and from rats deficient in the vitamin B complex, fixed and dehydrated by the freezing-drying method and stained with hematoxylin and eosin or the Giemsa stain, showed no changes.

The most pronounced changes were noted in peripheral nerves. Slight degeneration was apparent in the spinal cord.

CHANGES IN THE PITUITARY GLAND FOLLOWING TOTAL THYROIDECTOMY

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Changes in the pituitary gland in human spontaneous myxedema, consisting in hypertrophy and increase in weight, were first described by Niepcè, almost a century ago. The studies of Rogowitch on total ablation of the thyroid gland in animals, in 1888, further stimulated interest in the relationship between the thyroid and the pituitary gland. At the present time there is available an extensive literature concerning hypophysial changes both in spontaneous and in postoperative myxedema in man (table 1) and in myxedema thyreopriva of animals (table 2).

Blumgart and his associates have recently established the value of total thyroidectomy as a therapeutic measure in heart disease and other conditions; thyroidectomy has been performed in over a hundred patients. A number of these patients died following thyroidectomy and came to autopsy. The purpose of the present communication is to describe the pituitary glands of five such patients and to compare the changes found in them with those described in animals with experimentally induced myxedema and in patients with spontaneous and with postoperative myxedema.

MATERIAL AND METHODS

Studies were made on five patients who died within from four to nineteen months after total thyroidectomy. Four of the patients had received desiccated thyroid at various times.

The pituitary glands were weighed as soon as removed and at once fixed in Zenker's solution and solution of formaldehyde U. S. P. (1:10). Several sagittal sections 10 microns thick were taken from each pituitary gland; these were stained with the hematoxylin-eosin stain, the eosin-methylene blue stain or the special differential stain advocated by Spark.

REPORT OF CASES

CASE 1.—Total thyroidectomy was performed in this 34 year old Russian Jewish housewife in December 1933. Fifteen weeks later she commenced to show dryness of the skin and puffiness of the eyes. These gradually progressed so that

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TABLE 1.—*Pituitary Gland in Cases of Myxedema in Man in Which Autopsies Were Made*

Group	Author	Cases	Gross Description	Microscopic Description
Spontaneous:				
	Niepce	3	Enlarged; weight increased	
	Virchow	1	Pars distalis atrophied	
	Henrot	1	Enlarged	
	Barling	1	Normal	
	Bourneville and Briçon	1	Enlarged	
	Clinical Society of London	?	Normal	
	Pisenti and Viola	1		Cells vacuolated; colloid increased
	Duckworth	?	Normal	
	Ord	?	Normal	
	Shelswell	?	Normal	
	Whitwell	?	Normal	
	Whipham	?	Normal	
	Boyce and Beadles	3	Enlarged	Cells of pars anterior large and vacuolated; colloid increased
	Grøn	1	Enlarged	
	Burkhardt	1	Enlarged	
	Osler	1	Atrophied	
	de Coulon	5	Enlarged	Cells small; colloid increased; fibrosis
	Comte	1	Enlarged	Basophils decreased and vacuolated
	Maresch	1	Normal	Cells vacuolated; colloid increased
	Ponfick	1	Small	Cystic and fibrotic changes; many of the remaining cells large and full of colloid
	Aschoff	1	Enlarged	
	Packard and Hand	2	One normal One enlarged	
	Vassale	1	Enlarged	"Glandular hypertrophy"
	Rocaz and Cruchet	1	Normal	
	Abrikosoff	1	Enlarged	Cells large; chromophils vacuolated
	Bayon	1		Fibrosis; colloid increased; eosinophils increased
	Hunt	1	Enlarged	Normal
	Heyn	2*	Normal	Normal
	Dieterle	1	Normal	Normal
	Scholz	?	Normal	
	MacCallum and Fabyan	1	Enlarged	Basophils increased; eosinophils decreased; colloid increased
	Calderara	1	Enlarged, especially pars distalis	Basophils increased and vacuolated; colloid increased
	Sainton and Rathery	1	Destroyed by a cyst	
	Parhon and Golstein	1	Enlarged	
	Zieleniewska-Stéfanoff	2	Enlarged	Chromophils increased; colloid increased
	Schilder	6	Normal	Cells large and vacuolated
	Gaujoux and Peyron	1	Enlarged	Chromophils decreased; fibrosis
	Zuckerman	1	Enlarged	Chief cells hypertrophied
	Schultze	1	Enlarged	Cells large and vacuolated (like pregnancy cells)
	Eichhorst	1	Enlarged	Chronic inflammation; all cells small and degranulated
	Kraus and Holzer	1†	Weight increased	Chief cells large and vacuolated (like pregnancy cells)
Postoperative:				
	Schonemann	1	Enlarged	
	Garbini	1	Small	Cells large and vacuolated; colloid increased
	Langhans	1	Pars distalis enlarged	Chromophils increased
	Wegelin	1	Enlarged	Basophils vacuolated; fatty changes in other cells
	Schnitker, Van Raalte and Cutler	?	Normal	Normal

* Heyn presented a series of four cases, but two of these were instances of congenital syphilis rather than of myxedema.

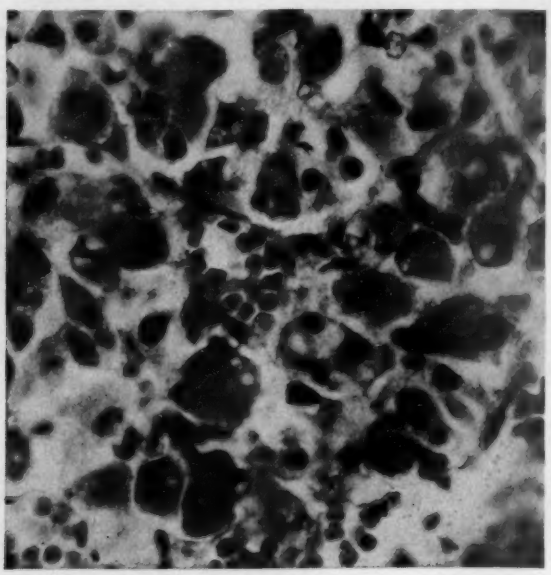
† This patient was pregnant.

TABLE 2.—*Pituitary Gland in Postoperative Myxedema in Animals*

Author	Animal Used	Gross Description	Microscopic Description
Rogowitch	Dog and rabbit		Chief cells large, vacuolated and full of colloid; eosinophils decreased
Tizzoni and Centanni	Dog	Normal	Like that given by Rogowitch
Stieda	Rabbit	Weight increased	Chief cells hypertrophied and vacuolated
Ewald and Rockwell	Dove	Normal	
Gley	Rabbit	Weight increased	
Schwarz	Dog		Like that given by Rogowitch
Vassale	Dog	Normal	Normal
Hofmeister	Rabbit	Weight increased	Chief cells hypertrophied and vacuolated
Rosenblatt	Dog	Pale and edematous	Chromophils increased; cells vacuolated
Blumenreich and Jacoby	Guinea-pig and goat	Normal	
Munk	Cat, dog, ape, rabbit	Normal	Normal
Leonhardt	Rabbit	Weight increased	All cells enlarged; chief cells vacuolated
Traina	Dog		Cells vacuolated; granules decreased
Katzenstein	Dog and ape	Normal	
Torri	Dog and rabbit		Chromophils, especially eosinophils, increased; colloid increased
Thaon	Sheep		Large cells vacuolated
Alquier	Dog	Enlarged	Eosinophils increased, hypertrophied and vacuolated
Cimoroni	Dog and rabbit	Enlarged	Basophils increased and vacuolated; eosinophils increased
Hagenbach	Cat	Pars distalis enlarged	
Lucien and Parisot	Rabbit	Enlarged	
Herring	Dog and rabbit		Pars distalis normal; colloid in pars nervosa; neuroglia hypertrophied
Walter	Rabbit	Enlarged	
Parhon and Golstein	Dog		Basophils increased; eosinophils decreased
Harvier	Cat		Chromophils and colloid increased
von Cyon	Rabbit	Enlarged	
Viguier	Lizard		Large vacuolated cells; basophils increased
Edmunds	Cat and dog	Normal	Normal
Livingston	Rabbit	Weight increased	
Degener	Rabbit	Weight increased	
Tatum	Rabbit	Enlarged	Large vacuolated cells
Gozzi	Dog		Chromophils and colloid increased
Falta	?	Normal	Normal
Trautman	Goats	Normal	Chief cells hypertrophied and vacuolated; eosinophils and basophils decreased
Kojima	Rat		Large vacuolated cells; colloid increased; changes irreversible
Hoskins and Morris	Frog	Normal	
Rogers	Frog	Enlarged	
Kamo	Dog	Pars distalis enlarged	Large vacuolated cells; chromophils decreased
Satwornitzkaja	Dog		Basophils increased and vacuolated; eosinophils decreased
Poos	Cat and dog	Enlarged	Chief cells hypertrophied and vacuolated
Evans and Simpson	Rat	Weight increased	
Bryant	Rabbit	Weight increased	Chromophobes increased and vacuolated; eosinophils decreased
Puglisse	Dog		Basophils increased; eosinophils decreased; chief cells hypertrophied and vacuolated; amphophilic cells appearing
Van Dyke and Ch'en	Rabbit	Weight increased	
Hohlweg and Junkmann	Rat		Large vacuolated cells; eosinophils decreased
Severinghaus, Smelser and Clark	Rabbit		Basophils increased; eosinophils decreased
Marine, Rosen and Spark	Rabbit		All cells increased; eosinophilic granules decreased; changes reversible
Zeckwer, Davison, Keller and Livingood	Rat	Weight increased	Basophils increased and vacuolated; eosinophils decreased

the skin became very dry and scaly, the face and hands very puffy, the speech slow and thick, and the hair coarse and dry. Seven months after operation the basal metabolic rate was minus 52 per cent. During the next four months it ranged between this level and minus 46 per cent. Seventeen months after operation use of desiccated thyroid, ½ grain (0.32 Gm.) three times a day, was started. The dose was reduced to ¼ grain (0.16 Gm.) three times a day one month later. Nineteen months after operation the patient died of pulmonary infection.

Autopsy was performed four hours post mortem. No remaining thyroid tissue was found. The sella turcica was not remarkable. The pituitary gland weighed approximately 1 Gm. The pars distalis appeared slightly enlarged. Surfaces made by cutting through it sagittally revealed a cellular, moderately firm yellowish brown tissue. The pars nervosa consisted of the usual firm, uniformly



Characteristic enlargement and vacuolation of basophilic cells of the pars distalis; eosin-methylene blue; × 630. Many of the vacuoles are poorly visualized because they are out of focus.

pale yellowish gray tissue. Microscopic examination revealed no abnormalities in the pars nervosa and pars intermedia. In the pars distalis the basophilic cells were strikingly prominent under low power magnification. They were distributed throughout the pars distalis. These cells appeared to be two or three times as numerous as in the average normal female pituitary gland. Under higher magnification most of the basophilic cells appeared larger than normal. The nuclei were round to oval; a few nuclei were pyknotic. The basophilic cells were from one to four times the size of the acidophilic cells. The cytoplasm of most of the basophilic cells was packed full of blue-staining granules. The cytoplasm of almost every basophilic cell contained from one to three small round vacuoles, which were either colorless or stained a faint grayish blue. Some vacuoles occupied one quarter of the cell body. The acidophilic cells were fairly uniformly dis-

tributed. The nuclei were round to oval; many were pyknotic. The cell membrane was distinct, and the cytoplasm was full of acidophilic granules. Very small vacuoles were found in a few acidophilic cells. These vacuoles, when present, nowhere reached the size or number found in any of the basophilic cells. In general, the acidophilic cells appeared to be smaller than normal, though no striking change in number was observed. The chromophobe cells were essentially normal in all respects. The stroma and blood vessels showed no abnormal changes.

Case 2.—This 31 year old Russian Jewish housewife entered the hospital in January 1934, complaining of dyspnea, orthopnea, palpitation and frequent hemoptyses for eleven years, and edema of the legs for two years. A diagnosis of rheumatic heart disease with mitral stenosis and insufficiency and auricular fibrillation was made. Total thyroidectomy was performed in January 1934. The basal metabolic rate fell rapidly, and eight weeks after operation puffiness of the eyelids and face and drowsiness appeared. At this time the basal metabolic rate was minus 29 per cent. Desiccated thyroid, ½ grain daily, was prescribed, with prompt disappearance of the evidences of myxedema and with a rise in the basal metabolic rate. The thyroid medication was discontinued after a week, following which the basal metabolic rate varied between minus 29 and minus 33 per cent. The serum cholesterol level on two occasions was 258 mg. per hundred cubic centimeters.

Beginning a few days after operation, the patient noted paresthesias in the fingers and face, which were found to be due to parathyroid insufficiency. Her symptoms were controlled with calcium chloride, taken by mouth, but recurred whenever she discontinued the use of this drug. It never became necessary to administer parathyroid extract.

Eight months after operation she reentered the hospital in marked congestive heart failure, following a severe infection of the upper respiratory tract. At this time she showed moderate generalized yellowish pallor and marked puffiness and dryness of the skin of the eyelids, face, hands, forearms and feet. There was also moderate thinning of the lateral third of the eyebrows. In spite of all treatment she became rapidly worse and died in a few days.

Autopsy was performed five hours post mortem. No remaining thyroid tissue was found. The sella turcica was not remarkable. The pituitary gland weighed 670 mg. The pars distalis appeared slightly enlarged, measuring 6 by 10 by 14 mm. Surfaces made by cutting through it sagitally revealed a very cellular, moderately firm yellowish brown tissue, yellower centrally and browner peripherally, with no definitely distinguishable stroma and with a few barely perceptible vessels scattered about. The pars nervosa appeared slightly hypertrophied, measuring 3 by 5 by 6 mm. It consisted of a firm, uniformly pale yellowish gray tissue.

On microscopic examination the pars nervosa of the pituitary gland revealed the usual structure. A few cystic structures small in size, lined by blue-staining cuboidal cells were found in the pars intermedia. Microscopic examination of the pars distalis showed the basophilic cells distributed throughout. The prominence of these cells under low power magnification was striking. They appeared to be from two to three times as numerous as in the average normal female pituitary gland; they were approximately from one to three times the size of the acidophilic cells. Almost every basophilic cell contained at least one vacuole; several of them contained as many as five. The acidophilic cells were fairly uniformly distributed; many were small, containing little cytoplasm. Very rare small vacuoles were present in a few of them. The chromophobe cells were not abnormal. The blood vessels and stroma showed nothing unusual.

CASE 3.—Total thyroidectomy was performed on this 58 year old Russian Jewish housewife in February 1934. Following operation the basal metabolic rate fell to minus 27 per cent. During the next two months she received between ½ and ¼ grain of desiccated thyroid daily. No thyroid was taken during the last six months of life, except during the three days immediately preceding death, when she again took ¼ grain daily. Moderate generalized pallor, puffiness and dryness of the skin of the eyelids, cheeks and hands, moderate thinning and dryness of the hair and some sluggishness were present after operation until the time of death. She died suddenly of coronary thrombosis eight months after operation.

Autopsy was performed eleven and one-half hours post mortem. No remaining thyroid tissue was found. The sella turcica was not remarkable. The pituitary gland weighed 800 mg. The pars distalis was hypertrophied and in the process of enlargement had apparently displaced the pars nervosa posteriorly and slightly upward. Surfaces made by cutting the gland sagittally showed the two lobes fairly well defined. The pars distalis consisted of a moderately firm yellowish brown tissue, browner peripherally and yellower centrally, with barely perceptible bright red points scattered about, representing blood vessels, about one to the square millimeter. The substance of the pars distalis appeared denser than normal. The pars nervosa was slightly hypertrophied and appeared firm and uniformly pale yellowish gray.

On microscopic examination the basophilic cells were found distributed throughout the pars distalis. They varied in size, shape and degree of granularity. Many basophilic cells contained only from 10 to 20 blue-staining granules, while others were packed full of them. Those with few granules were small and suggested that they might be chromophobe cells in the process of becoming basophilic cells. Many basophilic cells contained large pale blue hyaline vacuoles; most of the cells contained at least two or three. The basophilic cells, including those with few granules, were from three to four times as numerous as in the average normal female pituitary gland. The acidophilic cells varied in size and shape. Many of the larger acidophilic cells contained vacuoles. In this pituitary gland the vacuoles of the acidophilic cells were more prominent than in the other pituitary glands studied but were uniformly very much smaller and fewer than those found in the basophils. The chromophobe cells showed no abnormalities. The stroma and blood vessels were not remarkable. The pars intermedia and parts nervosa appeared quite normal.

CASE 4.—Thyroidectomy was performed on this 21 year old Italian in August 1933. The basal metabolic rate fell to minus 24 per cent, and the serum cholesterol level rose from 138 to 254 mg. per hundred cubic centimeters. The patient gained in weight and acquired a moderate yellowish pallor, dryness of the hair and slight puffiness of the skin of the eyelids and hands. He never received desiccated thyroid. Congestive cardiac failure recurred ten months after operation and did not respond to the usual therapeutic measures. He died in a few weeks.

Autopsy was performed three hours post mortem. No remaining thyroid tissue was found. The sella turcica was not remarkable. The pituitary gland weighed 650 mg. The pars distalis appeared slightly enlarged, measuring 5 by 9 by 15 mm. Surfaces made by cutting through it sagittally revealed a very cellular, moderately firm yellowish brown tissue, yellower centrally and browner peripherally, with no definitely distinguishable stroma and with a few barely perceptible vessels scattered about. The pars nervosa also appeared slightly hypertrophied, measuring 3 by 3 by 5 mm. It consisted of a firm, uniformly pale

yellowish gray tissue. The tissue was poorly preserved. On microscopic examination the pars nervosa and pars intermedia appeared normal. Examination of the pars distalis revealed the basophilic cells distributed throughout. Each contained from one to three vacuoles. The basophilic cells appeared to be one and a half or two times as numerous and from one to three times as large as in the average normal male pituitary gland. The acidophilic and the chromophobe cells showed no definite abnormality. The blood vessels and stroma were not remarkable.

CASE 5.—Total thyroidectomy was performed on this 50 year old Russian Jew in March 1936. The basal metabolic rate fell promptly and six weeks after operation reached a level of —25 per cent, about which it fluctuated during the rest of the patient's life. Nine weeks after operation puffiness of the eyelids was noted, and during the next month puffiness of the face and hands, yellowish pallor and huskiness of the voice appeared. His serum cholesterol rose from 205 to 301 mg. per hundred cubic centimeters three months after operation. Four months after operation he suddenly died of pulmonary edema.

Autopsy was performed ten hours post mortem. No remaining thyroid tissue was found. The sella turcica was not remarkable. The pituitary gland appeared normal in size and weighed 431 mg. The pars distalis measured 6 by 6 by 8 mm. and the pars venosa 3 by 3 by 4 mm. Sagittal section revealed no gross abnormality.

On microscopic examination the pars nervosa of the pituitary gland revealed no abnormalities. The pars intermedia contained a few small colloid-filled cysts. The basophilic cells of the pars distalis were two or three times the normal in number. Most of them were from slightly to markedly enlarged. Approximately half of them contained from one to four vacuoles, filled with a clear, glassy, pale blue material. In many instances the vacuoles were large enough to push aside or compress the nucleus. The eosinophilic cells were normal in size and appearance except that the nuclei in many of them were pyknotic. The chromophobe cells were not remarkable. The stroma and blood vessels were normal.

COMMENT

Although reported changes in the pituitary glands of patients with spontaneous myxedema (table 1) show considerable variation, they may be divided into two main groups: (1) atrophy or destruction of the parenchyma with or without a chronic inflammatory reaction and (2) hypertrophy of the pars distalis with the appearance of large pale vacuolated cells, which frequently contain colloid material.

The findings in the reported animal experiments (table 2) in which positive results were obtained are strikingly similar to those in the second group. The nature of the large vacuolated cells has been a matter of controversy, a variety of origins for these cells having been suggested. Some of the earlier authors regarded them as representing a new type of cell; others felt that they were derived from chief (chromophobe) cells. The most recent work seems to indicate that they are modified basophilic cells (table 2).

A decrease in size of the eosinophilic cells with loss of specific granulation has also been reported in many instances of spontaneous hypothyroidism in man and experimental myxedema thyreopriva in animals.

Indeed, some workers regard this as the essential change in the pituitary gland in myxedema. Others, however, regard it as a very late manifestation of complete myxedema.

Study of the cases in our series shows conclusively that the principal change in the human pituitary gland in postoperative myxedema is an increase in the number, size and degree of vacuolation of the basophilic cells. The number of basophilic cells was increased from two to four times the normal. Less striking was the inconstant slight diminution in size of the eosinophilic elements. The size and weight of the pituitary gland were increased in the four patients who died eight months or more after operation.

The increase in basophilic cells apparently occurs at the expense of the chromophobe cells. In one of the pituitary glands studied, scattered chromophobe cells were seen containing a few basophilic granules, suggesting that they were in the process of becoming basophilic cells. That the changes observed in the glands in our cases are not as marked as those found in some cases of spontaneous myxedema or in most of the animal experiments is possibly due to the fact that myxedema did not develop to an extreme degree, because of the administration of small doses of desiccated thyroid. Kojima, on the basis of a few experiments, concluded that the hypophysial changes in animals with induced myxedema could not be reversed by thyroid feeding, but the later, more extensive work of Marine, Rosen and Spark showed conclusively that such changes are reversible.

The significance of these changes cannot be stated with certainty. Increased amounts of thyrotropic hormone in the pituitary gland (Zeckwer), blood and urine in myxedema (Hertz and Oastler) have been reported. Whether or not the increase in the production of thyrotropic hormone is related to the increase in the number and in the degree of vacuolation of the basophilic cells is not yet known.

The observations on the pituitary glands of patients with postoperative myxedema suggest an explanation for the variable histologic changes in the hypophyses of patients with spontaneous myxedema reported in the literature. It is possible that in the reported instances of spontaneous myxedema in which the pituitary gland showed hyperplasia and vacuolation of the basophilic cells the hypothyroidism was due to primary atrophy of the thyroid gland, since this is the picture in the pituitary gland in postoperative myxedema in man as well as in lower animals. On the other hand, in those instances of human spontaneous myxedema in which the pituitary lesion was atrophic or degenerative in nature, the primary defect might have been in the hypophysis, atrophy of the thyroid gland with resultant myxedema being due to an insufficient production of thyrotropic hormone by the pituitary gland.

Changes in the pituitary gland of the patient (case 2) with chronic parathyroid insufficiency were indistinguishable from the pituitary changes in the other four patients.

SUMMARY

The pituitary glands in five patients with postoperative hypothyroidism were examined and were found to show an increase in number, size and degree of vacuolation of the basophilic cells. The number of these cells was increased from two to four times the normal. Less striking was the inconstant slight diminution in size of the eosinophilic elements. The size and weight of the pituitary gland were increased in the four patients who died eight months or more after total thyroidectomy; that of the fifth, who died four months after operation, was normal in size but showed the characteristic microscopic changes.

The changes observed resemble those found after total thyroidectomy in animals and correspond closely to those observed in some patients with spontaneous myxedema. Other patients with spontaneous myxedema show fibrosis and atrophy of the hypophysis. This suggests that spontaneous hypothyroidism in man may have more than one cause; in some patients it is due to atrophic changes in the pituitary gland, while in others it is caused by primary atrophy of the thyroid gland itself.

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ULTRAVIOLET SPECTROPHOTOMETRY OF BIOLOGIC FLUIDS

I. BLOOD PLASMA FOLLOWING IMMUNIZATION TO ALPHA CRYSTALLINE LENS PROTEIN

II. TETANUS AND DIPHTHERIA ANTITOXIC SERUM

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The failure to demonstrate specific changes in the ultraviolet absorption spectra of blood plasma obtained from patients with a variety of diseases [1] prompted us to determine what changes, if any, occurred in the blood plasma of the experimental animal following immunization. Because of the large amount of work involved, only one protein was studied. Alpha crystalline lens protein was selected because of Hektoen's [2] work on its potency as an antigen and because Poynter and Allen [3] used this protein and their experience was available to us.

METHOD

The lens protein was obtained from beef lens by the Woods and Burky method.[4] The immunizations were done according to the method used by Poynter and Allen and are described in detail by Young.[5] Briefly 3, 3.5 and 4.5 cc. of the aqueous solution of lens, containing 71.9 mg. of protein nitrogen per hundred cubic centimeters, were injected on the first, fourth and eighth days. Nine days after the last injection the blood samples were analyzed spectrophotometrically. The precipitin reaction was positive at a dilution of 1:16,600 in the case of rabbits B-1 and B-2 and at dilutions between 1:1,660 and 1:8,300 in the case of rabbit B-3. In the case of rabbits B-1 and B-2 complement fixation was 4+, and in the case of B-3, 3+, with the antigen diluted 1:80. The normal absorption spectra were determined over a period of four months prior to immunization, and in the case of B-4 this period was extended to six months. Food was withheld for twenty-four hours before cardiac puncture. The rabbits used remained healthy

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1. Dunn, F. L., and Sudman, A. T.: Arch. Path. **21**:463, 1936.
2. Hektoen, L., and Schulhof, K.: J. Infect. Dis. **34**:433, 1924.
3. Poynter, C. W. M., and Allen, E. W.: Am. J. Ophth. **8**:184, 1925.
4. Woods, A. C., and Burky, E. L.: J. A. M. A. **89**:102, 1927.
5. Young, R.: A Study of the Immune Body, Thesis, University of Nebraska, June 1930.

throughout the study. The spectrophotometric method has been described in our work on blood plasma.[1] The region included that from 2,100 to 3,500 angstroms, and this was extended to 6,500 angstroms in a few specimens. Heparin was the anticoagulant.

RESULTS

The results are shown in the charts. In chart 1 and in chart *2A* the absorption coefficient is calculated to 1 mm. cell depth, the plasma being undiluted. Chart *2A* is that of the normal rabbit B-4, the outer curve being the boundaries of all the curves obtained over a period of six months. The middle curve is a graphic average of the data. In chart 1 the solid line is the graphic average of the data obtained during the four months prior to immunization; the dotted line is the absorption

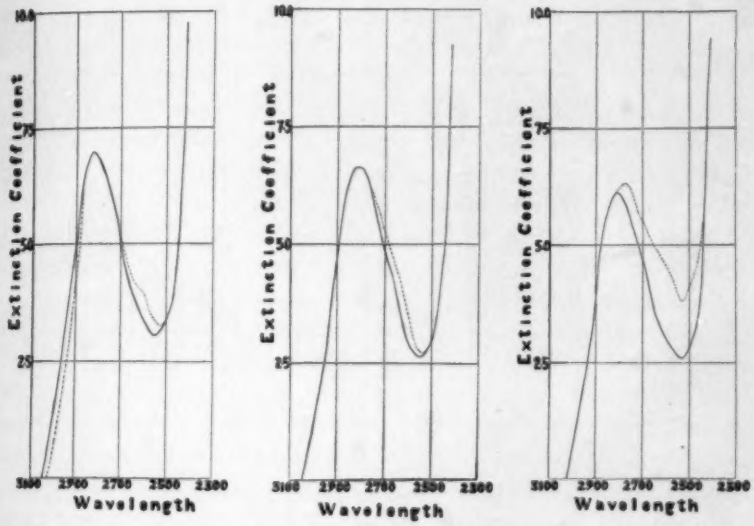

Chart 1.—Absorption curves of rabbits B-1, B-2 and B-3 (reading from left to right). The solid lines are graphic averages of the absorption curves obtained during a four month control period prior to immunization. The dotted lines are the absorption curves obtained following immunization to alpha crystalline lens protein.

curve following immunization. The postimmunization curve in B-3 was the only one that extended outside of the boundaries established prior to immunization. This was the animal with the lowest precipitin titer. Globulin fractions were prepared by adding 1 cc. of plasma to 9 cc. of five-ninths saturated ammonium sulfate, filtering and washing with half-saturated ammonium sulfate. The precipitate was completely redissolved in 15 cc. of water and photographed with a cell 5 mm. deep. No significant differences were noted as compared with globulin similarly prepared from normal plasma.

Chart *2B* shows the absorption curves of diphtheria antitoxic serum (Mulford M 6 purified globulin superconcentrated lot no. 26486-3) and

tetanus antitoxic serum (Mulford M 60 superconcentrated lot no. 27605-1). It was necessary to remove the phenolic preservative because of the intense absorption by such substances. This was done by precipitating the globulin and washing with ether until ether concentrates taken up in water gave only a faint blue with Folin's phenol reagent. The residue was then redissolved in water and photographed with a 2 mm. cell. The absorption constant is for a 2 mm. cell depth. The wavelengths of the maxima and minima are essentially identical for the two substances and correspond very closely with those reported by

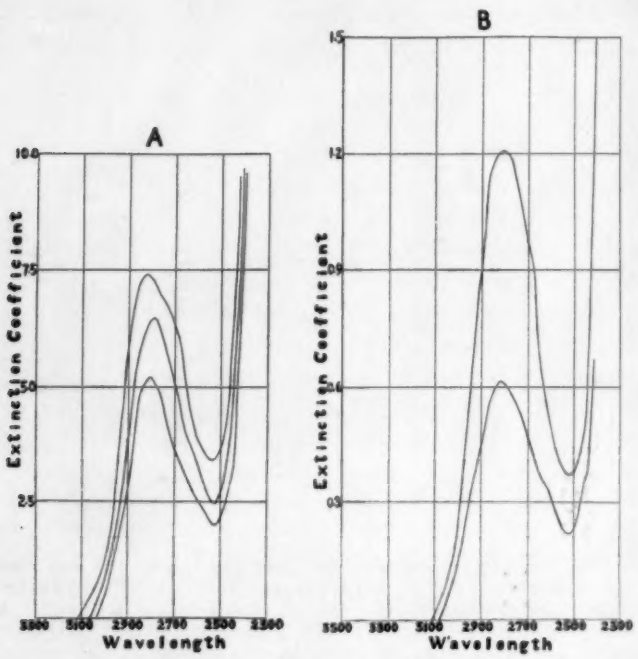

Chart 2.—In *A* are shown the absorption curves of rabbit B-4. The outer curves are the envelop of the absorption data obtained over a six month period. The inner curve is the graphic average. In *B* the upper curve is the absorption curve of diphtheria antitoxin and the lower curve, the absorption curve of tetanus antitoxin. The preservatives have been removed.

Marchlewski and Wierzuchowska [6] and by Svedberg and Sjögren [7] for serum globulin. The activities of these immune serums were not determined after the ether extractions necessary for the removal of the preservatives.

The absence of decisive specific differences in the absorption spectra within the range of the type of equipment used is in conformity with our studies on blood plasma in various diseases. Tadakora and

6. Marchlewski, L., and Wierzuchowska, J.: Bull. Internat. Acad. d. sc. de Cracovie, Math., nos. 9-10A, 1928, p. 471.

7. Svedberg, T., and Sjögren, B.: J. Am. Chem. Soc. **52**:2855, 1930.

Nakayama [8] observed changes in blood serum, using a dilution method. These they interpreted as being due to colloidal changes in the serum following immunization. Aside from the technical difficulties in using simple dilution methods for photometric measurements, it is almost safe to declare that changes in colloidal state affect the absorption spectrum in broad massive bands and not in narrow bands of absorption. Tadakora and Nakayama observed that the absorption spectra of immune serums "were much larger and longer than that of normal serum," a change which, Marrack [9] considered, is accounted for by the increase of serum globulin, which usually occurs on immunization. We did not observe this change. Pirosky [10] noted that the absorption curves of diphtheria and tetanus antitoxic serums are essentially the same. An abstract of a paper by Coulter, Kabat and Stone [11] reports that Felton's pneumococcic antibody solution and normal horse euglobulin gave identical absorption spectra.

It should be recognized that this report does not negative the possibility of demonstrating specific changes in absorption spectra of immune serums. Present methods of spectrophotometry represent marked advances over those available two decades ago. However, even at present it is difficult to maintain an accuracy of ± 2 per cent. At the same time photographic methods are less desirable than the continuous recording devices now becoming available in that the former may conceivably miss small changes in the slopes of absorption curves.

The absorption curve of blood plasma known at present deviates little if any in the position of its maxima and minima from that of serum globulin and serum albumin. Since the absorption curve of a solution is essentially a summation of the absorption curves of the individual substances present, it is perhaps not surprising that immunologic factors produce no recognizable changes in the protein curve.

SUMMARY

Following immunization with alpha crystalline lens protein the ultraviolet ray absorption of blood plasma in the rabbit does not deviate significantly from the normal.

The ultraviolet ray absorption of diphtheria and tetanus antitoxic serums resembles very closely the results reported by Svedberg and Sjögren and by Marchlewski and Wierzuchowska for serum globulin.

The ultraviolet ray absorption of blood plasma has been determined in the normal fasting rabbit over periods of from four to six months.

8. Tadakora, T., and Nakayama, Y.: J. Infect. Dis. **26**:8, 1920.

9. Marrack, J. R.: The Chemistry of Antigens and Antibodies, Medical Research Council, Special Reports Series, no. 194, London, His Majesty's Stationery Office, 1934, p. 55.

10. Pirosky, I.: Folia biol. **3-4**:9, 1934.

11. Coulter, C. B.; Kabat, E. A., and Stone, F. M.: J. Bact. **29**:54, 1935.

ENDOCARDIAL, ARTERIAL AND OTHER MESENCHYMAL ALTERATIONS ASSOCIATED WITH SERUM DISEASE IN MAN

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Proliferation of histiocytes in the subendothelial mesenchymal tissue of the mural and valvular endocardium has been noted in man and experimental animals under diverse conditions. The reaction has been most frequently encountered in scarlet fever (Fahr,[1] Siegmund,[2] Brody and Smith [3]) and is characterized by transformation of the loose undifferentiated endocardial layer into an edematous cellular structure composed of many histiocytic or monocytic elements. This alteration is generally most marked in the mural endocardium of the ventricles lining the trabeculae carneae, the mouths of the thebesian vessels and the chordae tendineae. Less often it is seen in the valvular endocardium. It may be focal or diffuse and is frequently accompanied by focal or diffuse infiltration of similar cells around blood vessels and between muscle fibers.

A similar stimulation of the endocardial mesenchymal layer has been observed less commonly in diphtheria complicated by streptococcic septicemia (Fahr [1a]) and in protracted generalized infections, such as subacute bacterial endocarditis, epidemic meningitis and staphylococcic septicemia (Siegmund [2b]).

Somewhat similar lesions have been produced in animals by injections of antigenic substances or other agents. Proliferation of the subendothelial mesenchymal cells has thus been observed in animals after repeated introduction of killed or virulent micro-organisms (Siegmund,[4] Dietrich [5]) or following the injection of vital dyes (Siegmund,[2b] Thom-

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1. Fahr, T.: (*a*) Virchows Arch. f. path. Anat. **232**:134, 1921; (*b*) Beitr. z. path. Anat. u. z. allg. Path. **85**:445, 1930.

2. Siegmund, H.: (*a*) Verhandl. d. deutsch. path. Gesellsch. **26**:231, 1931; (*b*) Virchows Arch. f. path. Anat. **290**:1, 1933.

3. Brody, H., and Smith, L. W.: Am. J. Path. **12**:373, 1936.

4. Siegmund, H.: Verhandl. d. deutsch. path. Gesellsch. **20**:260, 1925; footnote 2 *b*.

5. Dietrich, A.: München. med. Wchnschr. **78**:1328, 1928.

son [6]). Particularly striking, and more closely simulating the lesion in scarlet fever and in the cases to be described here, is the diffuse cellular thickening of the mural and valvular endocardium and of the intima of the aorta and pulmonary artery which has been observed in sensitized rabbits following intravenous injections of foreign serum (Vaubel,[7] Apitz,[8] Junghans [9]).

Following the suggestion that the morphologic alterations of rheumatic fever represent hypersensitiveness to streptococci or their products (Swift and others [10]), experiments of the latter type were undertaken. The lesions induced in experimental animals by prolonged anaphylaxis were compared with those occurring in rheumatic fever in man. Klinge [11] described widespread mesenchymal alteration in sensitized rabbits which received intra-articular injections of horse serum. The lesion was characterized by focal hyaline or fibrinoid necrosis of connective tissue, associated with proliferation of histiocytes, and occurred in joints, cardiac valves, myocardium, arteries, veins and skeletal muscle.

Klinge's report was followed by many other studies of the structural changes induced in animals by protracted anaphylaxis, and in most of them attention was directed chiefly to the alterations in the smaller coronary arteries and the periarterial mesenchymal tissue. Perivascular granulomas, fibrinoid swelling and necrosis, with cellular infiltration and endothelial proliferation, have been noted in the smaller coronary arteries in sensitized rabbits following introduction of horse serum (Vaubel,[7] Apitz,[8] Knepper and Waaler,[12] Murasawa [13]), bovine serum (Takeda [14]) and "specific antiheart serum" (Masugi, Sato and Todo [15]) and in guinea-pigs treated with horse serum (Ceroli [16]). The view has been frequently expressed that these arteritic and periarteritic lesions are morphologically similar to the spontaneous lesions which have been encountered in rheumatic fever, periarteritis nodosa, scarlet fever and

6. Thomson, J. G.: Beitr. z. path. Anat. u. z. allg. Path. **95**:316, 1935.

7. Vaubel, E.: Beitr. z. path. Anat. u. z. allg. Path. **89**:374, 1932.

8. Apitz, K.: Virchows Arch. f. path. Anat. **289**:46, 1933.

9. Junghans, E.: Beitr. z. path. Anat. u. z. allg. Path. **92**:467, 1933.

10. Swift, H. F.; Derick, C. L., and Hitchcock, C. H.: Tr. A. Am. Physicians **43**:192, 1928.

11. Klinge, F.: Beitr. z. path. Anat. u. z. allg. Path. **83**:185, 1930.

12. Knepper, R., and Waaler, G.: Virchows Arch. f. path. Anat. **294**:587, 1935.

13. Murasawa, S.: Tr. Soc. path. jap. **25**:206, 1935.

14. Takeda, K.: Tr. Soc. path. jap. **25**:194, 1935.

15. Masugi, M.; Sato, Y., and Todo, S.: Tr. Soc. path. jap. **25**:211, 1935.

16. Cerioli, E.: Profilassi **6**:221, 1933.

streptococcic infections in man (Klinge,[17] Metz,[18] Vaubel,[7] Siegmund,[2a] Semsroth and Koch,[19] Abrikossoff and Rudik [20]).

Besides the endocardial and vascular reactions, mesenchymal alterations in parenchymatous organs have claimed attention. In man, these have been noted most frequently in scarlet fever. Brody and Smith [3] recently reviewed the literature on the visceral pathologic changes in scarlet fever and have reported their own findings in a large group of cases. An interstitial mononuclear infiltration was found in from 75 to 95 per cent of livers, kidneys, adrenals and spleens and to a variable degree in other viscera. The evidence suggested to them that these lesions are the result of the action of a circulating toxin and that primarily they occur as changes in the walls of blood vessels with secondary infiltration into the adjacent interstitial tissues. Although they saw similar lesions in other infectious diseases, those alterations were of a lesser degree and invariably associated with streptococcic infection.

In Councilman's [21] paper, in which is described the lesion of acute interstitial nephritis, are mentioned cases of infectious diseases other than scarlet fever and diphtheria. Infiltration of the periportal tissue of the liver by mononuclear cells, plasma cells and eosinophilic or neutrophilic leukocytes is a common finding in infectious diseases (Marcuse,[22] Kahlstorf,[23] Kettler [24]), although Noble [25] reported the absence of any correlation between this alteration and generalized infection. Marcuse [22] found the lesion most often in scarlet fever, diphtheria and typhoid fever, and less frequently in measles, pneumonia and other infectious diseases. Proliferation or infiltration of histiocytic elements beneath the endothelium of the trabecular splenic veins is a prominent finding in scarlet fever (Brody and Smith [3]) and in cases of generalized sepsis (Siegmund [26]).

In experimental animals, focal perivascular and interstitial infiltration in various viscera by cells of the lymphohistiocytic series has been

17. Klinge, F.: Virchows Arch. f. path. Anat. **278**:438, 1930; **279**:1 and 16, 1931; footnote 11.

18. Metz, W.: Beitr. z. path. Anat. u. z. allg. Path. **88**:17, 1931-1932.

19. Semsroth, K., and Koch, R.: Krankheitsforschung **8**:191, 1930.

20. Abrikossoff, A. I., and Rudik, D.: Arch. internat. d. med. exper. **10**:303, 1935.

21. Councilman, W. T.: J. Exper. Med. **3**:393, 1898.

22. Marcuse, B.: Virchows Arch. f. path. Anat. **160**:186, 1900.

23. Kahlstorf, A.: Beitr. z. path. Anat. u. z. allg. Path. **78**:512, 1927.

24. Kettler, L.: Virchows Arch. f. path. Anat. **291**:706, 1933.

25. Noble, J. F.: Am. J. Path. **9**:473, 1933.

26. Siegmund, H.: Verhandl. d. deutsch. path. Gesellsch. **19**:114, 1923; footnote 4.

induced by several methods. Nye and Parker,[27] Klostermeyer,[28] Ehrich and Wohlrab[29] and Ehrich and Voigt[30] observed them after repeated injections of killed bacteria. Kuczynski[31] claimed to have produced the interstitial lesions of scarlet fever in the liver and kidneys of rabbits by intravenous introduction of living streptococci. Perivascular cellular reactions in the lungs, liver or kidney, induced by intravenous injections of streptococci after preliminary sensitization, have been described by Metz[18] and Hitchcock, Camero and Swift.[32] Although these responses have been observed without preliminary sensitization,[33] the latter serves to enhance the animals' reactivity to the antigen.[34] Proliferation of the lymphohistiocytic elements of the liver and kidneys has also been noted in protracted anaphylaxis of experimental animals,[35] but the reaction in the abdominal viscera is meager as compared with that in the heart.[35b]

It is curious that despite the frequent use of therapeutic horse serum, no analogous endocardial, arterial or other mesenchymal alterations for which foreign serum might be held responsible have been reported in man. Two cases in which such alterations were exhibited have recently been studied at necropsy in Bellevue Hospital. They are presented here, and the presentation is followed by an analysis of the considerations which support the genetic relationship of the therapeutic serums administered.

REPORT OF CASES

CASE 1.—A white man, 54 years old, was admitted to the Third (New York University) Medical Division of Bellevue Hospital on Dec. 24, 1936, complaining of weakness, dyspnea and swelling of the ankles of two weeks' duration. He had migratory polyarthritis at the age of 15 and gonorrhea and a chancre at 21.

Physical examination revealed evidence of congestive heart failure. The point of maximal apical impulse of the heart was in the sixth left intercostal space, outside the midclavicular line; the sounds were of poor quality and the pulmonary second sound was greater than the aortic second sound; a short systolic murmur was heard over the aortic area, and the ventricular rate was regular.

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27. Nye, R. N., and Parker, F.: Am. J. Path. **6**:381, 1930.

28. Klostermeyer, W.: Virchows Arch. f. path. Anat. **288**:703, 1933.

29. Ehrich, W., and Wohlrab, R.: Beitr. z. path. Anat. u. z. allg. Path. **93**:321, 1934.

30. Ehrich, W., and Voigt, W.: Beitr. z. path. Anat. u. z. allg. Path. **93**:348, 1934.

31. Kuczynski, M. H.: Klin. Wchnschr. **3**:1303, 1924.

32. Hitchcock, C. H.; Camero, A. R., and Swift, H. F.: J. Exper. Med. **59**: 283, 1934.

33. Nye and Parker.[27] Hitchcock, Camero and Swift.[32]

34. Ehrich and Wohlrab.[29] Ehrich and Voigt.[30] Hitchcock, Camero and Swift.[32]

35. (*a*) Longcope, W. T.: J. Exper. Med. **18**:678, 1913. (*b*) Sato, Y.: Tr. Soc. path. jap. **24**:297, 1934. (*c*) Vaubel.[7] Metz.[18] Apitz.[6]

The temperature was 100.6 F., the pulse rate 100, and the blood pressure, 150 systolic and 72 diastolic. The urine showed a trace of albumin. There were 5,200,000 red cells per cubic millimeter of blood, with hemoglobin 100 per cent, and 12,700 white blood cells, with 70 per cent polymorphonuclear leukocytes. The Wassermann reaction of the blood was negative.

The signs of congestive heart failure became more marked during the next few days but subsided rapidly following administration of digitalis. On Jan. 7, 1937, the patient experienced a chill, with pain in the right side of the chest and right upper quadrant of the abdomen and a nonproductive cough. The temperature rose to 103 F. and the white cell count to 21,000. There was dulness at the base of the right lung, with many fine moist râles. The sputum revealed pneumococci of type V. On the following day dulness developed over the lower lobe of the left lung, and intravenous administration of type V antipneumococcus serum, of the New York City Health Department, was begun. Daily doses of about 225,000 units (75 cc.) were given until January 15, the total dose being 1,800,000 units (600 cc.). Blood cultures taken on January 8, 13 and 16 were sterile after one hundred and twenty hours' incubation. On January 14 there appeared a generalized urticarial rash, which was extremely itchy. Four days later the rash was almost gone, but joint pains developed. Throughout this period the patient appeared critically ill, though signs of congestive heart failure were not prominent. Death occurred on January 18.

Significant Observations at Necropsy.—Heart: There was moderate hypertrophy and dilatation of all chambers, and the organ weighed 600 Gm. There was no significant alteration of the pericardium, of the valves or of the mural endocardium of the right auricle and ventricle. The left auricular endocardium was but slightly thickened. The mitral ring measured 12 cm. in circumference, and there were diffuse thickening and moderate retraction of the leaflets, but no loss of flexibility. The chordae tendineae were thickened. There was no alteration of the endocardium. The aortic leaflets showed mild thickening, slight rolling of the free margins and slight agglutination of the commissures. The aortic and mitral valvular endocardium appeared smooth and was entirely free from verrucae or other vegetations. The aortic ring was dilated, measuring 11 cm. in circumference. The coronary arteries showed mild focal atherosclerosis.

Aorta: Alterations characteristic of syphilitic aortitis were present in the ascending aorta; both the ascending and the descending aorta showed mild atherosclerosis.

Lungs: In addition to the changes of chronic passive congestion there were several recent infarcts, softened and organizing, in the lower lobe of the right lung and occluding thrombi or emboli in the vessels leading to these areas. The mediastinal nodes were large, soft and red.

Microscopic Observations.—Blocks of the viscera were fixed in solution of formaldehyde U. S. P. (1:10) and stained with hematoxylin and eosin. After fixation of the heart in Kaiserling's solution, the standardized blocks of Gross, Antopol and Sachs [36] from the valves and twenty-seven blocks of myocardium from various regions of the auricles and ventricles were placed in solution of formaldehyde, embedded in paraffin, cut and stained with hematoxylin and eosin and with Weigert's elastic tissue and Van Gieson's connective tissue stains. Further, the Gram-Weigert stain was employed on a few sections.

Myocardium: The most striking alteration was diffuse dense cellular thickening of the mural endocardium of the ventricles, particularly over the columnae

36. Gross, L.; Antopol, W., and Sachs, B.: Arch. Path. **10**:840, 1930.

carneae, the mouths of the thebesian veins and the chordae tendineae. The lining endothelial cells were sometimes intact, sometimes invisible. In some zones it was difficult to determine whether the lining cells were hypertrophied endothelial cells or histiocytes. There were edema and slight fragmentation of collagen, but fibrinoid change or thrombi were entirely absent. At least 95 per cent of the cells were of the character of mononuclear histiocytes, showing clear cell outlines, varying in diameter from 10 to 30 microns, oval, polyhedral or fusiform, possessing abundant lightly basophilic cytoplasm and large centrally or eccentrically situated, coarsely chromatic nuclei. A few lymphocytes and eosinophilic polymorphonuclear leukocytes were also noted (fig. 1 *A*, *B* and *C*).

A widespread focal infiltration of identical cellular character between the muscle fibers and around the interfascicular capillaries and venules was encountered in sections of the right and left ventricular walls (fig. 1 *C* and *D*). There were also noted moderate nuclear and fiber hypertrophy and focal interfascicular and perivascular fibrosis with muscle replacement.

The periarterial and periarteriolar tissue was comparatively free from cellular infiltration or other alteration. A few foci were encountered which exhibited a sparse infiltration of histiocytes with or without interfibrillar edema. There was found only one periarterial focus possessing fragmented collagen. This was situated in the adventitia of a coronary artery in the pericardial wedge and appeared as a poorly demarcated zone of fragmented, lightly eosinophilic collagen infiltrated by one large and several small histiocytes and a few lymphocytes (fig. 2 *A*).

The small coronary arteries showed slight intimal thickening and medial fibrosis. Several were found displaying elevation and disruption of the endothelial lining by accumulations of subendothelial histiocytes and these were also found in the lumen (fig. 2 *B*). One of the larger coronary arteries was the seat of advanced atherosclerosis, and beneath the endothelial lining of a plaque was a broad dense band of histiocytes (fig. 2 *C*). Thrombi were uniformly absent.

Valves: The mitral leaflets showed dense hyaline fibrous thickening at the annulus, the latter possessing many thick-walled and thin-walled vessels and a few collections of histiocytes. The leaflets were diffusely thickened by dense fibrous tissue, and thick-walled vessels were noted throughout their length. In many sections the body of the leaflet was acellular or showed only a few lymphocytes and fibrocytes, but beneath the surface, on all aspects, was a broad dense diffuse layer of histiocytes, which descended beneath the endothelium of the thickened chordae tendineae (fig. 2 *D*). The lining endothelial cells were not visible. This superficial proliferation and infiltration was accompanied by edema, but collagenous necrosis, fibrinoid change or vegetations were not found. In other sections, in addition to the endocardial alteration, an intense perivascular histiocytic proliferation was found in the basal portion of the body of the leaflet, accompanied by separation of the collagenous and muscle elements of these thick vessels and occlusion of their lumens by masses of histiocytes and few endothelial cells (fig. 3 *A*).

The aortic valve showed diffuse acellular fibrous thickening and a few vessels in the basal portion. There were no vegetations. Histiocytic proliferation was not seen. The tricuspid leaflets showed a few histiocytes beneath the endothelium of the ventricular aspect. The pulmonary leaflets were unaltered.

Aorta: The ascending aorta exhibited adventitial thickening, perivascular lymphocytic infiltration, medial scarring and vascularization and hyaline intimal thickening, the whole quite characteristic of syphilitic aortitis. Beneath the endothelium were several focal and diffuse collections of histiocytes similar to those seen in the mural endocardium and coronary arteries. This reaction was seen in sections of the aorta in which signs of chronic syphilitic aortitis were absent.

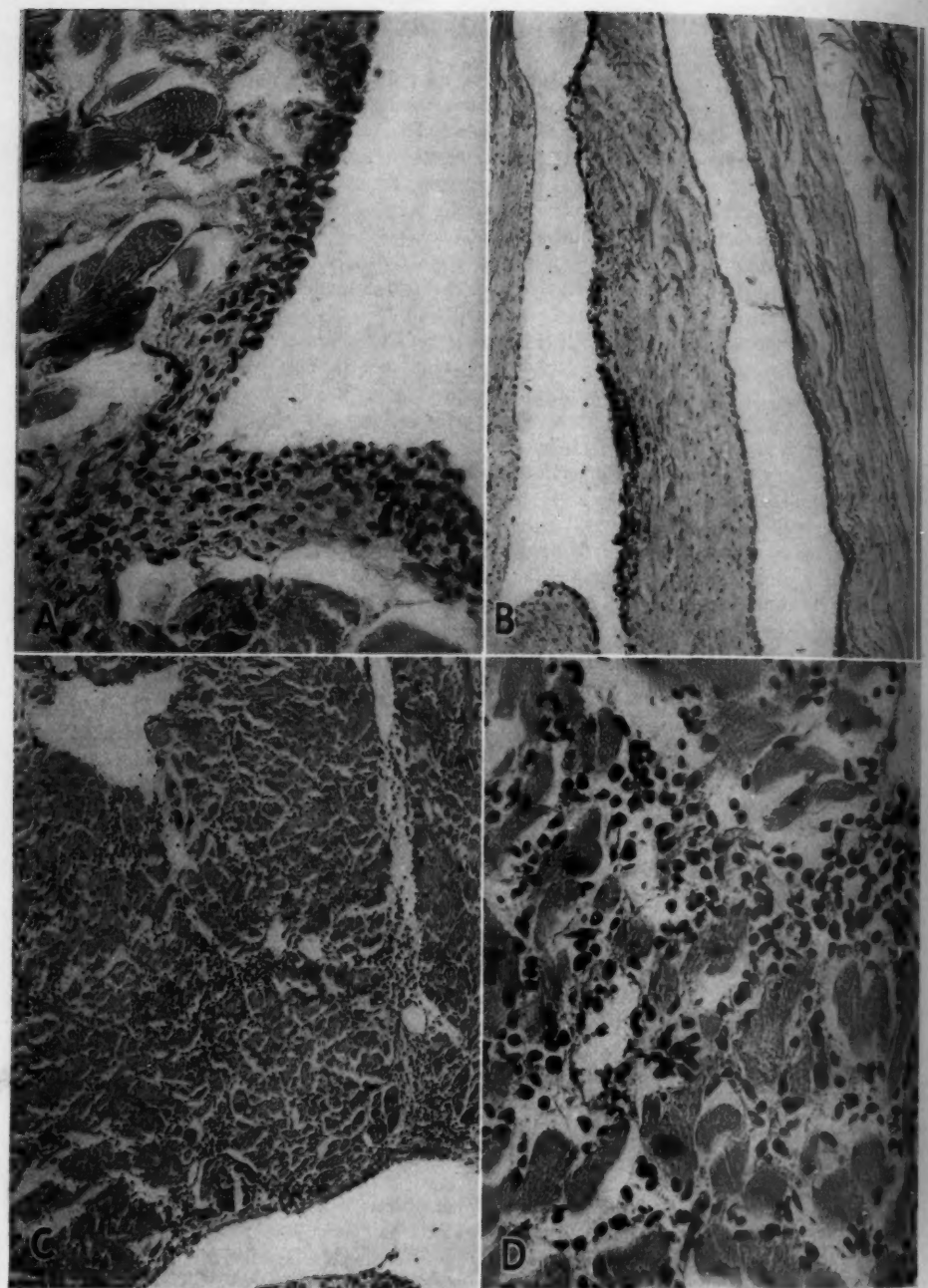

Fig. 1 (case 1).—*A*, section of the wall of the left ventricle, showing cellular thickening of the mural endocardium. *B*, view of the subendothelial band of histiocytes in the chordae tendineae. *C*, section of the left ventricle. Note the cellular thickening of the lining of the thebesian veins and the interstitial myocardial infiltrations. *D*, detailed view of the interstitial myocardial infiltration of histiocytes.

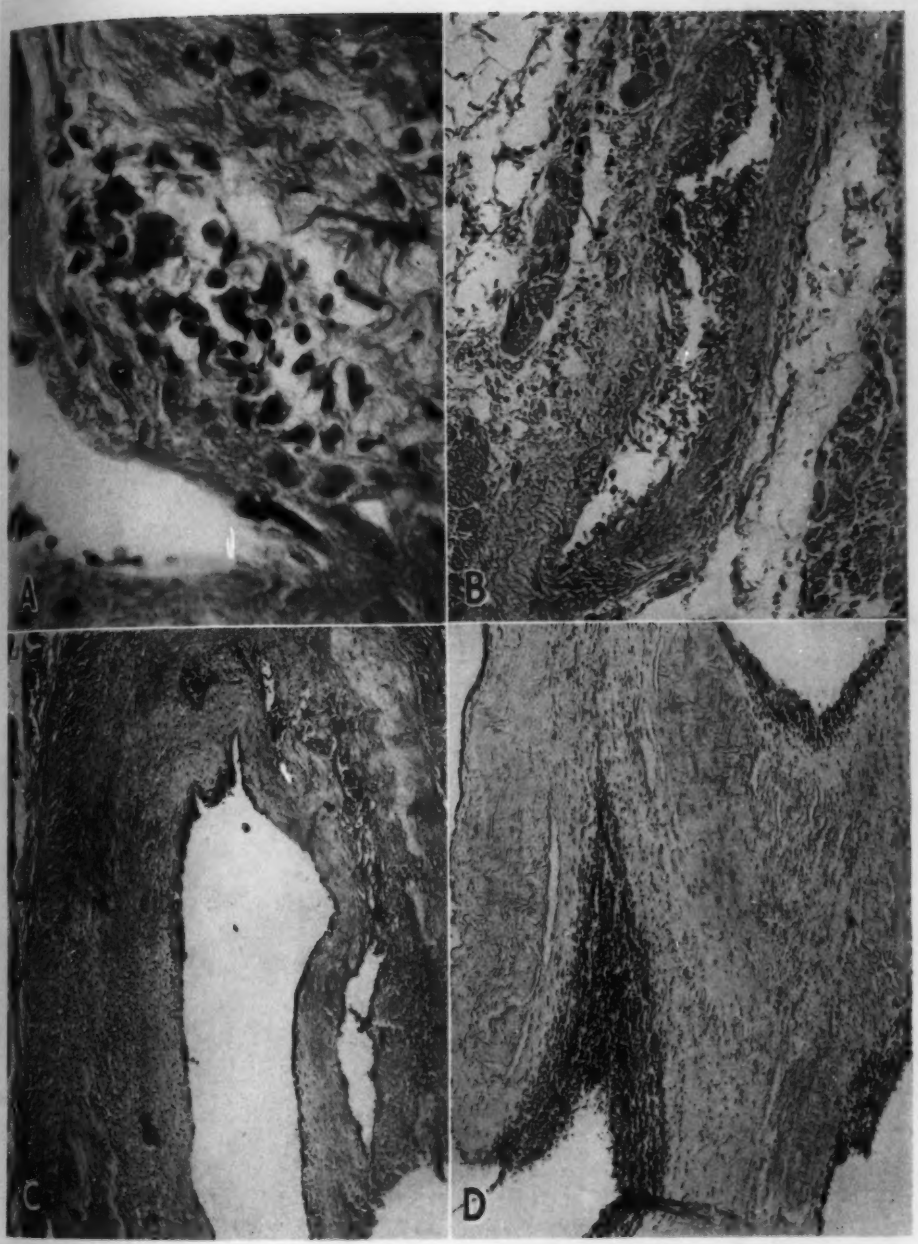

Fig. 2 (case 1).—*A*, detailed view of a nodule composed of fragmented collagen, histiocytes and lymphocytes in the adventitia of a coronary artery. *B*, section of a small coronary artery displaying marked proliferation of histiocytes, which partially fill the lumen of the vessel. *C*, section of a large coronary artery which possesses a broad superficial band of histiocytes. Note the absence of thrombotic deposits. *D*, section of the mitral leaflet, which shows diffuse fibrous thickening and a broad dense superficial layer of histiocytes. Note the absence of vegetations.

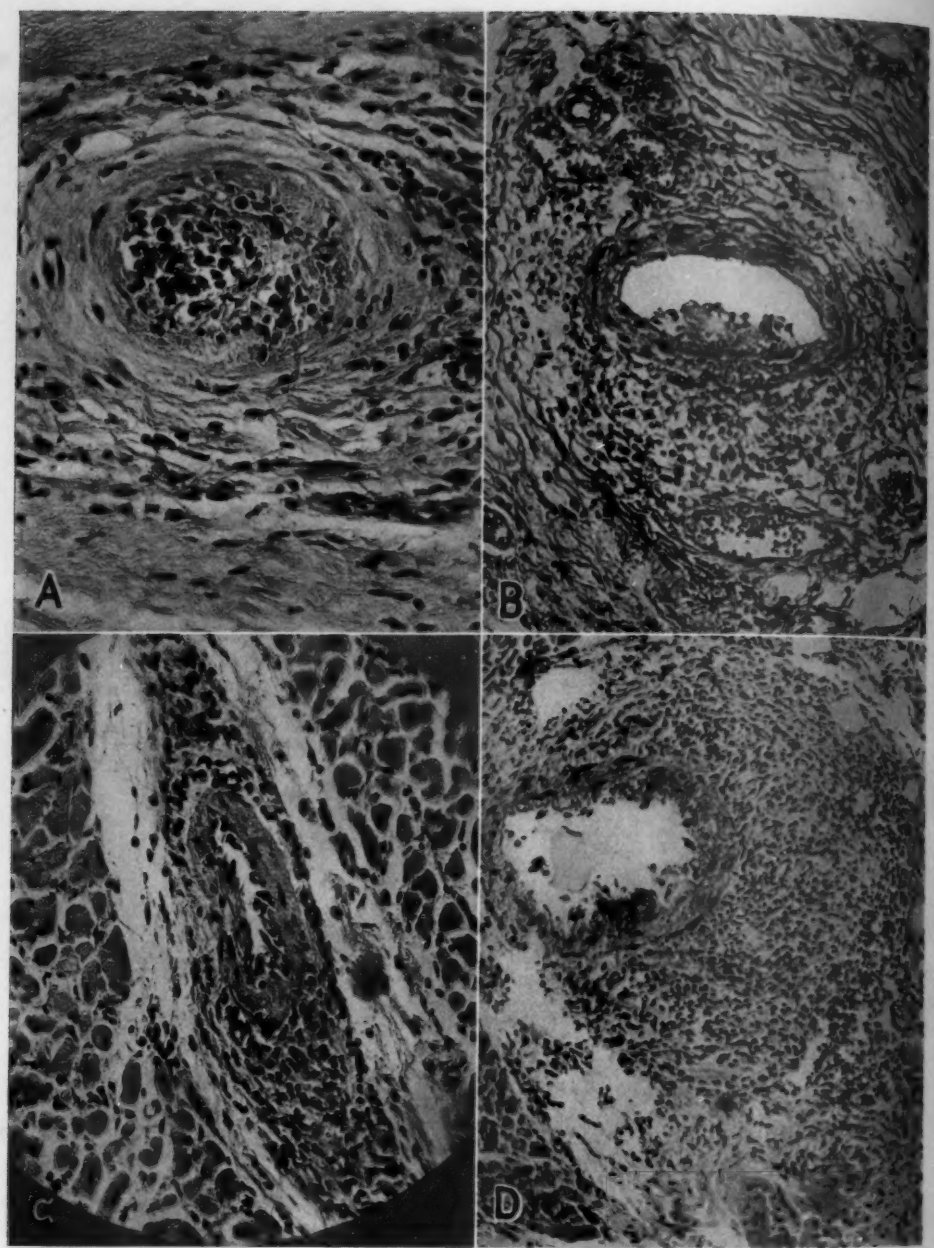

Fig. 3 (case 1).—*A*, section through the basal portion of the posterior mitral leaflet, showing a vessel occluded by histiocytes and perivascular accumulations of similar cells. *B*, section of the testis showing focal necrosis of the wall of a small artery, histiocytes in the lumen of the vessel and perivascular edema and histiocytic proliferation. *C*, section of the left ventricle showing a well developed perivascular granuloma. *D*, section of the left ventricle exhibiting homogeneous fibrinoid necrosis of the wall of a small coronary artery and a huge periarterial granuloma.

Other Viscera: There were focal collections of histiocytes, lymphocytes and eosinophilic leukocytes in the adrenal medulla and pericortical areolar tissue, in the perivascular tissue of the lungs and intima of the pulmonary arteries, in the portal areas of the liver, the intertubular tissue of the testes and the interstitial tissue of the renal cortex and the renal pelvic fat. In the testes, one small artery was seen which exhibited periarterial histiocytic infiltration, eccentric medial necrosis and histiocytes in its lumen (fig. 3 *B*).

Case 2.—A 47 year old white man was admitted to the Third (New York University) Medical Division of Bellevue Hospital on Oct. 31, 1935, complaining of pain in the right side of the chest of eighteen hours' duration. Early in the morning of the day of admission, he awoke with severe pain in the lower region of the chest, on the right, associated with chill, cough and blood-streaked sputum.

Physical examination revealed consolidation of the lower lobe of the right lung. The heart was not enlarged; there were no murmurs, and the rate was regular.

The temperature was 102.4 F.; the pulse rate was 132, and the respiratory rate 46 per minute.

During the first twenty-four hours, 100,000 units (100 cc.) of Lederle antipneumococcus serum, types I and II, was given intravenously. The patient experienced an immediate reaction following each administration of serum, characterized by flush, dyspnea and chill. The reactions were controlled by epinephrine. Within twenty-four hours there appeared a diffuse urticarial rash. On the day of admission, the sputum showed pneumococcus type VIII, and the blood culture, which was taken on admission, revealed 1 pneumococcus colony per cubic centimeter. Antipneumococcus serum type VIII, of the New York City Health Department, was given in large doses, totaling 1,900,000 units (2,000 cc.), during the next eleven days, averaging about 150,000 to 200,000 units daily. A second blood culture was made on the third hospital day but was found sterile after one hundred and twenty hours' incubation. The blood was cultured again on the fifth, seventh, twelfth and nineteenth days and was constantly sterile. On the tenth day after the initial dose of serum there appeared an urticarial rash and generalized stiffness of the arms and legs. Serum therapy was stopped the second day following the appearance of serum sickness. Toward the end of the second week there was evidence of spread of consolidation to the upper lobe of the right lung. During the third week there were signs of resolution at the base of the right lung, but the patient became progressively weaker. The white cell count varied between 28,000 and 40,000, with a marked shift to the left; the red cell count dropped to 1,400,000, with hemoglobin 23 per cent; the temperature fluctuated between 99 and 102 F. During the fourth week, there was further clearing of the signs in the right side of the chest, but the patient remained dyspneic, continued to lose ground and required repeated transfusions of blood. On the evening of November 29, thirty days after admission, he had a profuse hemorrhage of dark red blood from the nose and mouth and died soon afterward.

Significant Observations at Necropsy.—Heart: The organ weighed 380 Gm. The mural and valvular endocardium presented no alteration other than mild thickening of the free border of the anterior mitral leaflet. There were no vegetations or thrombi. The myocardium appeared uniformly brown, and the coronary arteries showed mild atherosclerosis. The pericardlium was natural.

Lungs: There was a cavity with soft, gangrenous walls which occupied about two thirds of the upper and middle lobes of the right lung and was filled with fresh clotted blood. An eroded vessel was noted coursing through the cavity. Patchy pneumonia was present in the lower lobe of this lung.

Microscopic Observations.—Sections were fixed, cut and stained as in case 1. Thirty-seven blocks from various regions of the auricles and ventricles were taken.

Heart: The cellular thickening of the mural endocardium was similar to that in case 1, but much less intense and more focal. The interstitial myocardial infiltration was also similar, and although widespread, sparser. Spindle-shaped histiocytes, fibroblasts and lymphocytes were more numerous than in case 1.

The most striking lesions were associated with small intramyocardial arteries. These presented the form of perivascular granulomas, without or with necrotizing arteritis. The former generally appear as dense periarterial and adventitial collars of histiocytes, fibroblasts, lymphocytes, eosinophilic and neutrophilic polymorphonuclear leukocytes and plasma cells embedded in faintly eosinophilic fragmented collagen (fig. 3 *C*). All gradations of perivascular involvement were found, varying from the presence of a few histiocytes and eosinophils, eccentrically related to a coronary arteriole or small artery, to a large granulomatous lesion, forming a complete collar about the vessel. Necrosis of the arterial walls, destroying muscle and elastic fibers and infiltrated by pyknotic cells, was noted in many lesions (fig. 3 *D*). These necrotic areas were homogeneous in appearance and deeply eosinophilic and revealed an affinity for dinitrophenol. The lumens of the vessels were patent in some instances but almost occluded by subendothelial histiocytic proliferation in others (fig. 4 *A*).

Small collections of histiocytes were seen beneath the endothelium on both aspects of the tricuspid, aortic and mitral valves, and a few similar cells were seen deeper within the leaflets. The latter showed no other alteration.

Aorta: Besides the presence of atherosclerotic plaques, there were noted in the ascending aorta collections of histiocytes within the adventitia and the outer half of the media, arranged about the capillaries. However, the most striking lesion was in the intima, where edema and subendothelial infiltration of histiocytes were marked (fig. 4 *B*).

There were focal collections of histiocytes, lymphocytes and eosinophilic polymorphonuclear leukocytes in the interstitial tissue of the kidneys and adrenals, beneath the endothelium of the trabecular splenic veins and in the portal zones of the liver.

SUMMARY OF REPORT OF CASES

There are described here the cases of two patients who received intravenously large volumes of antipneumococcus horse serum and in whom death occurred four and nineteen days, respectively, after the appearance of symptoms of serum disease. In case 1 the major pathologic changes observed were those of chronic inactive valvular disease of rheumatic type and pulmonary infarcts. In case 2 there were postpneumonic cavitation and pulmonary hemorrhage. Blood cultures were constantly sterile in case 1. In case 2, although the first culture yielded a single colony of Pneumococcus, several subsequent cultures remained sterile.

The distinctive structural change, common to these two cases, was proliferation of histiocytes in the mural and valvular endocardium, in the intima of the aorta and that of the pulmonary artery and in the interstitial tissue of the myocardium, liver, kidneys and adrenals. Accompanying this cellular reaction was focal edema of the supporting

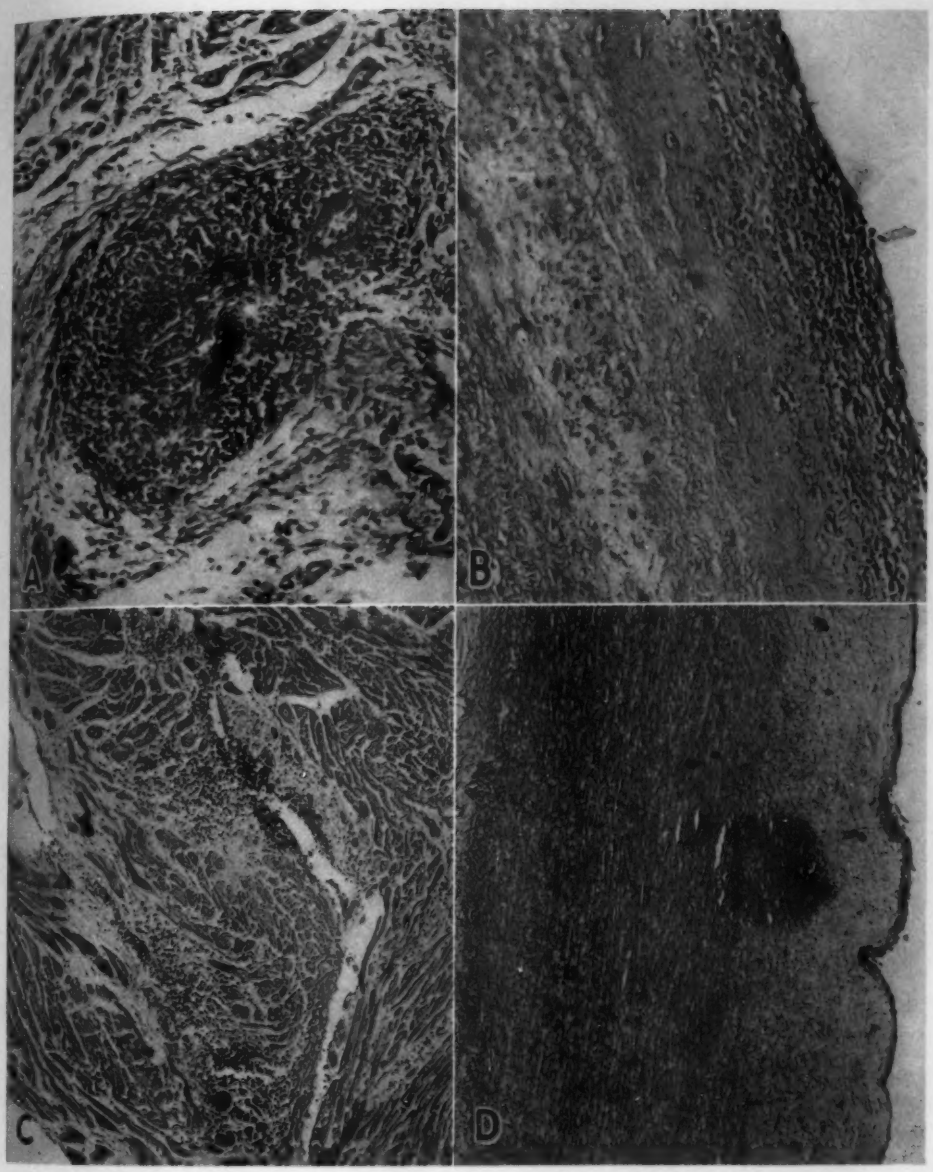

Fig. 4.—*A*, another example of necrotizing arteritis and periarterial inflammation (case 2). The lumen of the vessel is occluded by histiocytes. *B*, section of the aortic intima in case 2, showing marked superficial edema and infiltration of histiocytes. *C*, section of the left ventricle of a serum-treated patient with meningococcic meningitis, showing deposits of histiocytes lining the thebesian vessels and in the interstitial myocardial tissue. *D*, section of the aorta of a serum-treated patient who had meningococcic meningitis, showing a superficial band of histiocytes. The dark spherical zone is a staining artefact.

tissue and sparse infiltration by eosinophilic leukocytes and lymphocytes. Vegetations and Aschoff nodules were absent.

In both cases inflammatory arterial and periarterial lesions were found. In case 1, there was intense proliferation of the subendothelial histiocytes in the coronary arteries. One instance of focal periarteritis was found in the myocardium, and necrotizing arteritis and periarteritis were encountered in the testes. In case 2, the arterial alterations were more marked and appeared as perivascular granulomas, necrotizing arteritis and subendothelial histiocytic proliferation, limited to the smaller branches of the coronary arteries. There were swelling and fragmentation of collagen in the periarterial granulomas, but elsewhere collagen appeared unaltered.

The lesions in case 1 were composed chiefly of mononuclear histiocytic cells of oval or polyhedral type. The older age of the alterations in case 2 was reflected in the pleomorphic character of the cells and the presence of numerous spindle-shaped histiocytes and fibroblasts.

IDENTIFICATION OF THE LESION

In determining the nature of the alterations in these cases, consideration was given to all conditions in which similar lesions have been heretofore reported. As previously stated, it is believed that similar mural endocardial changes have been described in man only in association with scarlet fever and generalized sepsis. The clinical records of the two cases at Bellevue Hospital yield no support for the consideration of the former, and the persistently negative blood cultures exclude the latter.

That this reaction is not usually encountered in pneumococcic pneumonia, even in the presence of generalized sepsis, is indicated by the results of our examination of a series of fifty-two cases of this disease in which autopsies were made, in twenty-eight of which blood cultures were positive. Twenty-nine of the patients died without receiving serum, and one of these patients who had pneumococcic septicemia exhibited endocardial alteration of the type under consideration in a mild form. Infiltration of lymphocytes and histiocytes was found in the adrenals in four subjects, in the liver in one and beneath the endothelium of the trabecular splenic veins in eight. In twenty-three serum-treated patients who died before the development of serum disease, cellular alteration of the mural endocardium was not found. Mild focal proliferation of the valvular mesenchymal tissue was limited to two subjects, one with acute and the other with subacute vegetative endocarditis. Focal interstitial infiltrations were seen in the liver or adrenal in three cases and in the trabecular splenic veins in one instance. Arterial lesions were absent. Although in many

instances only one section of the mural endocardium and myocardium was available for examination, it is to be noted that in cases 1 and 2 of this report the significant lesions were discovered in routine sections, and numerous subsequent sections displayed the characteristic alterations in every instance.

Serious consideration was given to the possible rôle of rheumatic fever in the genesis of these lesions. In case 1, intense superficial histiocytic proliferation occurred in a leaflet which showed evidence of old rheumatic injury. The recognition that proliferation of the subendothelial mesenchymal cells sometimes represents an early stage in rheumatic valvulitis (Leary,[37] Jaffé,[38], Siegmund [2b]) led us to an evaluation of the various findings which might support or oppose the latter diagnosis. The architectonics of the cellular proliferation in case 1 did not correspond with those observed in the early reaction of rheumatic valvulitis, which, according to Leary,[37] is characterized by the formation of an orderly palisade of elongated ovoid or pyriform cell bodies arranged perpendicularly to the surface. The degree of cellular multiplication, the diffuseness and extent of the process in the heart, blood vessels and viscera, and the absence of vegetations of any type or of structures which closely resemble Aschoff bodies, lent strong support to the view that these alterations were not those of rheumatic activity.

In case 2, neither the history nor the gross observations at necropsy offered any evidence suggesting rheumatic fever. Only the lesions related to the smaller branches of the coronary arteries might raise the question of rheumatic etiology. Although some similarity exists between these vascular and perivascular alterations and those of rheumatic fever, no interstitial or perivascular structures were found which displayed close correspondence with the Aschoff nodule in any stage of its development (Aschoff,[39] Klinge,[17] Gross and Ehrlich [40]). Neither the other alterations in this case nor the absence of verrucae was in harmony with the usual histology of rheumatic fever.

Although syphilitic aortitis was present in case 1, there was no similarity between the changes in the heart and other viscera and those attributable to syphilis (Saphir,[41] Richter [42]). Nor were the edema and bandlike dense subendothelial proliferations of histiocytes of the aortic intima reminiscent of syphilitic aortitis.

37. Leary, T.: Arch. Path. **13**:1, 1932.
38. Jaffé, R. H.: Virchows Arch. f. path. Anat. **287**:379, 1932.
39. Aschoff, L.: Verhandl. d. deutsch. path. Gesellsch. **8**:46, 1904.
40. Gross, L., and Ehrlich, J. C.: Am. J. Path. **10**:467 and 489, 1934.
41. Saphir, O.: Arch. Path. **13**:266 and 436, 1932.
42. Richter, A. B.: Am. J. Path. **12**:129, 1936.

Hence it appears that the structural alterations in the two cases described here cannot be referred to those diseases in which similar changes have been hitherto reported. The association of serum disease with these alterations and their striking similarity to the structural changes induced in animals by protracted anaphylaxis led to the implication of the therapeutic serums in the genesis of the lesions.

RÔLE OF SERUM SICKNESS

With recognition of the etiologic importance of serum, the question arises whether this reaction is the direct effect of foreign serum or whether it represents the morphologic equivalent of a hyperergic state clinically associated with serum disease.

Of the twenty-three serum-treated pneumonic patients who were studied at necropsy and who had not shown serum sickness, twenty-one died from one to seven days after the initial dose of serum. These subjects received from 20 to 500 cc. of serum in from one to seven days. Two other patients lived more than seven days after the initial dose of serum. One of these received a single injection of 20 cc., died seventeen days later and showed no significant lesions. The other died with acute bacterial mitral endocarditis nine days after the initial dose of serum and showed only slight histiocytic proliferation of the aortic valvular endocardium. Endocardial, myocardial or arterial alterations did not develop when death occurred within a period of seven days after the initial dose of serum.

Four patients who received antipneumococcus serum came to necropsy after the development of serum sickness. Two of these were the patients concerned in cases 1 and 2 of this report. The third (V. C.) received serum early in the course of the disease and serum sickness developed nine days later. Empyema followed, the blood culture became sterile, and administration of serum was discontinued. It was resumed almost four weeks later because of the appearance of pneumococci in the blood cultures, and serum sickness appeared the day before death. Although some degree of endocardial and interstitial myocardial proliferation was found at necropsy, the occurrence of protracted sepsis and subacute bacterial endocarditis does not permit the implication of serum sickness.

The fourth (K. C.) received serum for pneumonia, and serum sickness developed. Convalescence was interrupted by the appearance of staphylococcic septicemia, followed several weeks later by hemolytic streptococcic septicemia and acute endocarditis. Death occurred more than two months after the serum sickness; mesenchymal alterations were not detected at necropsy.

Further information concerning the rôle of foreign serum or serum disease was sought in the examination of seventeen patients with meningitis who were treated with antimeningococcus serum and who came to necropsy at Bellevue Hospital within the past three years. In most instances only a single section of the left ventricle and other viscera was available for examination. Thirteen subjects, who received from 20 to 360 cc. of serum in from one to six days, died within from one to seven days after the initial dose of serum without serum disease developing, and no significant alterations were found, even though meningococcemia existed in three of them. In four other cases, serum disease appeared before death. Alterations were absent in two, in whom death occurred in twelve days after the disappearance of signs of serum disease. In one other, who died five days after serum disease appeared, slight focal mural endocardial and interstitial myocardial proliferation of histiocytes was observed. In the fourth, well marked lesions were encountered.

The latter received 265 cc. of antimeningococcus serum intraspinally during a period of twelve days. Meningococci were cultured from the spinal fluid, but four blood cultures remained sterile after one hundred and twenty hours of incubation. Serum disease developed ten days after the initial dose of serum, and death occurred two days later. Microscopic study of the organs revealed proliferation of histiocytes in the mural endocardium, interstitial myocardium, intima of the aorta and pulmonary arteries, liver, kidney and spleen (figs. 4 *C* and *D*). Inflammatory lesions of the coronary arteries or periarterial tissue were not encountered. Convincing implication of the administered antimeningococcus serum is precluded in this case because of the reported spontaneous appearance of somewhat similar lesions in meningococcic meningitis (Westenhoeffer,[43] Gruber,[44] Siegmund [2b]), although, the clinical protocols of such reports in most instances indicate the septicemic type of disease.

It is thus apparent that the distinctive changes exhibited in cases 1 and 2 do not constantly accompany serum disease. The factors which determine their appearance cannot be defined. The elicitation of these lesions may depend on the character of the foreign serum, the route and duration of administration or the state of reactivity of the subject. In the latter connection it is important to point out that in case 2 immediate reactions accompanied the initial and subsequent injections of serum, and a diffuse urticarial rash appeared within twenty-four hours after the first injection. It is possible that in case 1 a state of heightened tissue reactivity, referable to previous attacks of rheumatic

43. Westenhoeffer, M.: Deutsche med. Wchnschr. **32**:179, 1906.

44. Gruber, G. B.: Beitr. z. path. Anat. u. z. allg. Path. **61**:236, 1916.

fever, might have contributed to the intensity of the response. It has been shown that an allergic irritability of a nonspecific nature exists in subjects with rheumatic fever, even when this is inactive (Derick and Swift;[45] Swift, Derick and Hitchcock;[10] Swift[46]). Consideration that the alterations herein described have not been observed in serum-treated patients without clinical evidence of hypersensitivity and that preliminary sensitization is necessary for the elicitation of these lesions in serum-treated animals leads us to suggest that in cases 1 and 2 of this report these alterations represent a hyperergic reaction due to foreign serum. Whether they are a reaction of defense, representing the structural equivalent of the elaboration of antibodies or the parenteral digestion of foreign protein, or whether they reflect tissue injury due to antigen-antibody union is a subject unfortunately limited to speculation.

SIGNIFICANCE OF THE LESIONS HEREIN DESCRIBED IN THE PATHOGENESIS OF RHEUMATIC FEVER AND PERIARTERITIS NODOSA

Many writers have held the view that the lesions of rheumatic fever reflect an allergic type of tissue reaction.[47] Support for this view has been sought in studies of the mesenchymal alterations induced by prolonged anaphylaxis in animals. The morphologic identity of such experimental interstitial myocardial lesions with those of rheumatic fever has been asserted by Klinge[11] but denied by others (Aschoff,[48] Fahr,[1b] Saphir and Wile,[49] Masugi and others[15]). It has been suggested that the dissimilarity in the histology of the experimental and spontaneous lesions might be attributable to inherent differences in the reaction of different species. If the tissue changes in the two cases reported here are hyperergic, it must be admitted that the morphologic alterations of rheumatic fever are not identical with those observed in one form of hyperergic reaction in man. It thus becomes evident that this study cannot support the assertion that the characteristic lesion of rheumatic fever, i. e., the Aschoff body, represents merely a hyperergic reaction toward a nonspecific cause.

It has also been maintained that the characteristic vascular lesions of periarteritis nodosa represent allergic arteritis.[50] Striking similarities

45. Derick, C. L., and Swift, H. F.: Proc. Soc. Exper. Biol. & Med. **25**:222, 1927.

46. Swift, H. F.: J. Lab. & Clin. Med. **21**:551, 1936.

47. Siegmund.[2b] Swift, Derick and Hitchcock.[10] Klinge.[11] Leary.[37]

48. Aschoff, L.: Verhandl. d. deutsch. path. Gesellsch. **24**:13, 1929.

49. Saphir, O., and Wile, S. A.: Am. Heart J. **9**:1, 1933.

50. Rössle, R.: Virchows Arch. f. path. Anat. **288**:730, 1933. Vaubel.[7] Klinge.[11] Metz.[18]

are apparent between the arterial lesions in the heart in case 2 and the testes in case 1 and those sometimes observed in periarteritis nodosa. However, inferences concerning the pathogenesis of the latter disease which are based only on the morphologic resemblance between the two are of dubious value and are not included within the purpose of this report.

SUMMARY

In the foregoing report are presented the clinical and morphologic findings in two subjects who received large doses of antipneumococcus serum. The common distinctive structural alterations in these cases consisted of proliferation of histiocytes in the mural and valvular endocardium and in the intima of the aorta and the pulmonary and coronary arteries, accompanied by stimulation of the interstitial mesenchymal tissue of the myocardium and other viscera. Necrotizing arteritis and periarteritis of the smaller coronary arteries formed a prominent finding in one case (case 2). It is recognized that these lesions are not specific, and the diseases in which their occurrence has been previously reported are discussed. It is felt that the composite picture presented by these two cases is incompatible with any disease hitherto described. The coexistence of these lesions with clinical evidence of hypersensitiveness to foreign serum and their striking similarity to those induced in animals by protracted anaphylaxis lead us to suggest that these alterations are hyperergic and related to the administration of foreign serum.

HISTOLOGIC STUDY OF NUCLEI IN SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

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The purpose of this study is to examine histochemically the possible variations from the normal in the nuclei of the squamous cells of carcinoma of the uterine cervix and, by the same method, to study the effect of roentgen and radium irradiation on these nuclei.

Since the publication of Feulgen's [1] study more interest has been centered on the histochemical characteristics of nucleic acid (one of the primary constituents of nucleoproteins). Of all the chemical compounds found in cells, probably none are more important than these nucleoproteins. Wells [2] has stated that "nucelo-proteins are considered to be the most important constituents of the cell, both in quantity and in relation to cell activity." The basis of Feulgen's study is the reaction that takes place between decolorized basic fuchsin and nucleic acid. The procedure, if properly carried out, is specific for nucleic acid and results in the formation of a purple compound which is insoluble in water and only partly soluble in alcohol. The depth of the stain varies directly with the amount of nucleic acid present. According to Ludford,[3] "the general consensus of opinion is that the method provides a precise microchemical colour reaction."

MATERIALS AND METHODS

Twenty-one cases of squamous cell carcinoma of the cervix uteri were studied, five before, nine after, and seven both before and after, irradiation. The radiation consisted of roentgen rays alone in all except four cases, in which there was additional radiation therapy by means of radium. The amount of roentgen radiation given was from 900 to 2,000 roentgens (r), skin dose, with backscattering, focused on the cervix through two or more ports. The physical factors of measurement were as follows: 200 kilovolts (peak voltage) cross arm rectification, 0.5 mm. copper filtration, 60 cm. target skin distance, 25 milliamperes, half value 0.88

This study was made on a grant from Mrs. Lillie Schuster.
From the Research Department of the Barnard Free Skin and Cancer Hospital.

1. Feulgen, R., and Rossenbeck, H.: Ztschr. f. physiol. Chem. **135**:203, 1924.
2. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925, p. 19.
3. Ludford, R. J.: Proc. Roy. Soc., London, s.B **102**:397, 1928.

copper, 33 r per minute in air, divided doses. The four radium applications varied between 5,400 and 5,800 milligram hours. Sections of the irradiated tissue were taken from two to eight weeks after irradiation.

All tissues were fixed in a saturated solution of mercuric chloride in absolute alcohol, cleared in xylene, embedded in paraffin and stained according to the technic described by Ludford.[4] For purposes of comparison, sections from the same blocks were stained alternately by the Feulgen reaction and with hematoxylin and eosin.

HISTOLOGIC DATA

The nucleic acid demonstrable in the cells making up the normal squamous epithelium of the cervix uteri is present in the nuclear membrane and within the nucleus. The nuclear membrane takes a purple stain and is present in all nuclei except those of mitotic cells, in which the nuclear membrane is always absent. The nucleic acid within the nucleus of the normal resting cell usually stains either as multiple dark spots, arranged just within and close to the nuclear membrane, or as irregular broad bands of a lighter purple, indistinctly outlined, throughout the nucleus. As a general rule, the contents of the smaller, more active-appearing nuclei stain more darkly and sharply than do the seemingly less active larger nuclei. When the cell is in mitosis, the intranuclear contents stain still more darkly and sharply. With the alinement of the chromosomes in the metaphase, the nucleic acid stains its darkest purple.

In addition to the usual types of nuclei described in the foregoing paragraph, we noted three other variations in the distribution of nucleic acid in the cells that made up the normal epithelium of the uterine cervix. In the first type the nucleic acid collected into a dark homogeneous mass against one side of the nuclear membrane as in a typically pyknotic cell. In the second variation, the nuclei were enlarged and less deeply stained, and showed a more even distribution of the nucleic acid throughout the nucleus. In many nuclei of these two types the nuclear membrane did not seem to be intact. The pyknotic type was the much more frequent of the two. The third and least common aberration, noted in the nonmalignant cells, was that of nuclei containing vacuoles, which varied in size up to the point of occupying the entire nucleus. These vacuoles also varied in position. Some were central, while others were eccentric. Those located eccentrically were usually found in nuclei of the elongated type and were frequently large enough to distend and distort one end of the nucleus. Occasionally two or three of these vacuoles appeared in a single nucleus. As one focused on these areas, they all seemed to be spherical and void of any nucleic acid. Little could be learned of the nucleolus except that it took a very dark Feulgen stain. This stain also revealed that nucleic acid was frequently found extranuclearly as irregular homogeneous dark-staining masses.

The appearance and distribution of nucleic acid in malignant cells were the same as in the cells of nonmalignant tissue. In other words, the Feulgen stain failed to reveal any differential characteristics between normal and malignant cells. As in normal tissue, the nuclei of the apparently more active cells were smaller and stained more darkly and sharply than those in less active cells. Comparisons

4. We feel that it is wise, as recommended by Kelley and Miller (J. Biol. Chem. **110**:113, 1935) and others, to fix the tissues for the Feulgen stain by placing the specimens in mercuric chloride in absolute alcohol. The staining itself is easily done with a little practice. Our slides were very satisfactory from the point of view of both detail and permanency. We have observed sections for a period of two years after preparation, and none have showed any signs of deterioration.

of the amounts of nucleic acid demonstrable by this stain in the nuclei of normal and malignant cells were, of necessity, based on a gross judgment rather than on measurements of any exactitude. However, it seemed that both types showed in comparable areas about the same amounts of nucleic acid. The three variations in distribution of nucleic acid noted in the normal epithelium, namely, the pyknotic, the enlarged and the intranuclear vacuolation type, were also found in the cells comprising malignant tissue. Nucleoli and extranuclear distribution of nucleic acid appeared to be the same in tumor tissue as in normal tissue.

In sections stained by the Feulgen method, after irradiation, changes of the same types were noted in the nuclei both of the epithelial cells of the normal cervix uteri and of those comprising the tumors; these changes were, however, more pronounced in the tumor tissue. The amount of postirradiation reaction varied with the radiosensitivity of each tumor, and, to a lesser degree, with the quantity of radiation administered.

The most striking change following irradiation, as seen with the nucleic acid stain, was an edema-like swelling of some nuclei. In these nuclei the nuclear membrane stained more lightly and indistinctly, appearing as a pink blurred outline instead of the normal sharp purple line. Frequent breaks were noted in these nuclear membranes. The nucleic acid in the enlarged nuclei also stained much lighter than normally and appeared as irregular pink blurs instead of as the darker spots or bands described. An increase in the number of pyknotic nuclei was also noted following irradiation. These, however, did not seem to be as numerous in the tumor tissue as nuclei of the edematous or enlarged type. In three cases, the most notable change following irradiation was the presence of many vacuolated nuclei. The growths in all three of these cases received relatively small amounts of roentgen radiation, and all showed little effect from the therapy other than the increased vacuolation.

The Feulgen stain failed to reveal any configuration of nucleic acid which might serve as an index to radiosensitivity before radiation is administered. Neither did this stain disclose any criteria which would aid in grading the malignancy of a tumor. The latter statement is made with reference to the routine comparison of alternate sections stained with Feulgen and with hematoxylin and eosin. The Feulgen stain, however, did seem to possess one distinct advantage over the hematoxylin and eosin stain. This difference was noted in comparing sections taken before and after irradiation. The Feulgen stain always revealed more nuclear damage than did the hematoxylin and eosin stain in tissues subjected to the same dose of radiation.

COMMENT

Histologically we could see no qualitative difference between the nucleic acid in normal tissue and that in carcinoma. Klein and Beck[5] have stressed the chemical similarity between the nucleic acid from normal and that from malignant tissues. On the other hand, Stern and Willheim[6] found that nucleic acid prepared from the neoplastic tissues in cases of malignant disease had a lowered nitrogen content and an altered ratio of potassium to nitrogen when compared with the usual nucleic acid.

5. Klein, G., and Beck, J.: Ztschr. f. Krebsforsch. **42**:163, 1935.
6. Stern, K., and Willheim, R.: Biochem. Ztschr. **272**:180, 1934.

Microscopic comparisons of the quantitative variations of nucleic acid in the different nuclei were very difficult to evaluate accurately in our experiments. This was due to the fact that the nucleic acid seemed to be more concentrated, and therefore to stain more deeply, in the smaller, more active nuclei, whereas in the larger, less active nuclei, the nucleic acid was more evenly distributed and gave a lighter stain. As the distribution of nucleic acid in malignant and in normal tissue was of the same type, we were unable to make accurate comparisons of differences in amounts of nucleic acid. Ludford [3] observed Feulgen stains on experimentally produced tar tumors and was also unable to determine any apparent differences in the amounts of substance stained in the malignant nuclei and nuclei of the immediately neighboring normal skin. Cowdry,[7] however, stated that nuclei of rat sarcoma cells contained more nucleic acid than did normal rat fibroblasts. We were able to make the definite observation that there was a marked decrease in the amount of nucleic acid in the large edematous nuclei present after irradiation.

More exact methods of determining nucleic acid are desirable, for it seems logical to assume that quantitative variations occur in a substance so intimately connected with nuclear activity. Caspersson [8] studied nucleic acid in nuclei, using the ultraviolet spectrum and checking with chemical analysis. He found that in the resting nucleus there was a "haphazard" distribution of substances rich and poor in nucleic acid, but that during mitosis the nucleic acid seemed to localize in the chromosomes. He also determined that the nucleic acid content of the nucleus increased shortly before mitosis and decreased after. It was his opinion that a certain quantity of nucleic acid was necessary for mitotic division. Some of these findings of Caspersson are in keeping with our observations.

Following irradiation an increase in the number of aberrant nuclear forms was, of course, to be expected. As stated earlier, the aberrant nuclei varied in type; in normal epithelium those of the pyknotic type were more prominent, whereas in tumor tissue the enlarged, edematous nuclei were more numerous. In a previous paper [9] on the early effects of irradiation on normal epithelium, we described a sequence of changes in which we thought pyknosis followed swelling of the nucleus. With the Feulgen stain, it seemed that these changes were typical of the normal epithelium. In the malignant cells, however, it seemed that the nuclei enlarged and became edematous, with dilution and lighter

7. Cowdry, E. V.: Science **68**:138, 1928.

8. Caspersson, T.: Chem. Abstr. **30**:8267, 1936.

9. Seelig, M. G.; Eckert, C. T., and Cooper, Z. K.: Am. J. Cancer **25**:585, 1935.

staining of the nucleic acid. It also seemed, with the Feulgen technic, as already stated, that the nucleic acid tended to disappear from these swollen nuclei. The latter phenomenon was strikingly demonstrated by the Feulgen reaction. It is interesting to note that many years ago Ewing,[10] in speaking of this swelling of the nuclei as "one of the strictly specific features of the cell damage from radiation," stated that "one may suggest that it consists in an imbibition of water by the nucleoproteins."

We cannot explain the vacuolation of some of the nuclei. Roskin,[11] who also described the distribution of nucleic acid with the Feulgen stain, mentioned these vacuoles but did not attempt to interpret them. We may suggest only that they are forms of localized degeneration of the nucleus. The presence of extranuclear nucleic acid was clearly shown with this stain. Roskin [11] called these particles extruded chromatin, whereas Ludford [3] thought they were nucleolar extrusions. From our sections we were unable to determine the source of these particles, although many of the degenerative nuclei had definite defects in the nuclear membrane.

In comparing the Feulgen stain with the ordinary hematoxylin and eosin stain, it appeared that the former was of much greater value in demonstrating the nuclear changes following irradiation. We were able to define more extensive changes after irradiation in the Feulgen stains. This difference is probably due to the fact that by this stain we can more accurately follow changes in one of the most important components of the radiosensitive part of the cell, the nucleus.

CONCLUSIONS

The Feulgen stain will not differentiate the nuclei of squamous cell carcinoma of the uterine cervix from the nuclei of adjacent normal squamous epithelium.

The distribution of nucleic acid, as demonstrated by the Feulgen method, is the same in nuclei of squamous cell carcinoma of the cervix as in nuclei of adjacent normal squamous epithelium.

Following irradiation, the types of nuclear changes demonstrated by the Feulgen reaction are the same in squamous cell carcinoma of the cervix as in the adjacent normal squamous epithelium.

More nuclear changes are seen following irradiation in sections stained by the Feulgen method than in comparable sections stained with hematoxylin and eosin.

10. Ewing, J.: Am. J. Roentgenol. **15**:93, 1926.
11. Roskin, G.: Ztschr. f. Krebsforsch. **22**:472, 1925.

FATAL NICOTINE POISONING

A REPORT OF TWENTY-FOUR CASES

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The reported instances of fatal nicotine poisoning are relatively infrequent, as evidenced by Webster's [1] mention of only twenty-two cases. Among these are the five described by McNally [2] in 1921 and seven additional cases reported by him [3] a year later. McNally's descriptions are the most complete of any that have appeared in recent years. His method of isolating, identifying and quantitatively estimating nicotine is the one we have followed in our laboratory for a number of years. While Franke and Thomas [4] were able to collect seventy instances from the literature, in the great majority of these death followed the use of tobacco infusions and enemas; they add four examples occurring in St. Louis over a ten year period. Cowburn [5] described three instances in England. Lawes [6] in reporting two cases stated that nicotine poisoning in Australia is rare. Other scattered reports are found throughout the literature. According to the United States Department of Commerce, Bureau of the Census,[7] the following deaths from nicotine compounds have been reported in the United States Registration Area:

Deaths	1930	1931	1932	1933	1934
Suicidal	24	30	46	35	47
Accidental	13	9	8	35	41
Yearly total	37	39	54	70	88
Grand total	288				

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1. Webster, R. W.: Legal Medicine and Toxicology, Philadelphia, W. B. Saunders Company, 1930, p. 618.

2. McNally, W. D.: J. Lab. & Clin. Med. **5**:213, 1920.

3. McNally, W. D.: J. Lab. & Clin. Med. **8**:83, 1922.

4. Franke, F. E., and Thomas, J. E.: J. A. M. A. **106**:507, 1936.

5. Cowburn, A. D.: Med.-Leg. & Criminol. Rev. **1**:118, 1933.

6. Lawes, F. A. E.: M. J. Australia **1**:84, 1928.

7. Department of Commerce, Bureau of Census: Personal communication to the authors.

In Portland, Ore., over a ten year period there have been twenty-two deaths directly attributable to the ingestion of nicotine insecticides; two other cases, one in a nearby city in Washington, and the other in the southern part of Oregon, have come to our attention, bringing the total to twenty-four. In no instance has nicotine alkaloid or the pure sulfate preparation been used, to our knowledge.

Prior to 1927, the records of the Multnomah County coroner and of the department of pathology of the University of Oregon Medical School make no mention of nicotine as a lethal agent. At this time there occurred two deaths almost simultaneously and from the same source; the attendant newspaper sensationalism is in part responsible for the immediate and continued popularity of nicotine compounds as suicidal agents in this community. The ready availability of nicotine insecticides probably plays the more prominent rôle. Black Leaf 40, a commercial tobacco by-product containing 40 per cent nicotine sulfate and 60 per cent "inert ingredients," is widely used in this locality for killing aphids, a common parasite of the rose plant, and can be purchased without restriction at almost any store. It is produced commercially by steam distillation of tobacco with subsequent addition of sulfuric acid to form nicotine sulfate.[8]

Nicotine ranks fourth among the different poisons responsible for accidental, homicidal and suicidal deaths in Portland over a ten year period ending in September 1936. The percentage for each type of poison (statistics of the Multnomah County coroner) is as follows:

	Cases	Percentage
Carbon monoxide	126	49.5
Alcohol (acute alcoholism)	35	13.7
Phenols and cresols	31	12.2
Nicotine	22	8.6
Barbiturates	14	5.5
Cyanide	11	4.3
All others	16	6.2

Since nicotine products have assumed a rôle of importance as a cause of death in this region, and further because there are so few authentic reports concerning them on record, the following account seems advisable. The table briefly summarizes the available physical data concerning these deaths.

We are aware that in some of our cases absolute proof of nicotine poisoning was not established, but in those instances in which an autopsy was not performed, the finding of brownish material in the mouth with

8. Tobacco By-Products Company: Personal communication to the authors.

Summarized Data on Twenty-Four Cases of Fatal Nicotine Poisoning

Case	Status*	Sex	Age	Amount, Cc.	Fatal Period‡	Autopsy	Comment
1	A	M	34	30?	2 min.	+	Drank from whisky flask; died en route to lavatory
2	A	F	35	30?	3-10 min.	..	Drank from same flask as patient in case 1; collapsed in three minutes; dead within ten minutes
3	S	F	30	30?	?	+	Drank whisky glassful in a suicide pact; a drunken companion vomited, recovering
4	S	F	47	30?	?	..	Found dead in hotel room with empty 1 ounce (31.1 cm.) bottle;† suicide note present
5	S	M	?	30?	?	..	Found dead in rooming house with an empty 1 ounce bottle beside him
6	S	M	39	30	5 min.	..	Stepped into a store holding up empty 1 ounce bottle, remarked, "I just drank this, send for my wife," and collapsed. Dead on arrival at hospital
7	S	M	50	60	?	..	Found dead with two empty 1 ounce bottles beside him; a suicide note was present
8	?	M	44	?	?	+	A known chloroform addict found dead in a hotel room with no nicotine preparations nearby; death was thought to be due to chloroform poisoning until toxicologic analysis (Dr. H. B. Myers) revealed nicotine "in considerable quantity" along with chloroform
9	S	M	57	30	1 min.	..	Came home intoxicated and following a domestic argument drank a 1 ounce bottle; is thought to have died almost immediately
10	S	M	57	30	?	..	Found dead in rooming house with empty 1 ounce bottle; a suicide note was present
11	S	F	38	30	10 min.	+	Drank 1 ounce bottle; ran 50 feet (15 meters) and collapsed; dead within ten minutes
12	S	F	16	30	10 min.?	..	Drank an unknown amount; died before aid could be summoned
13	S	M	32	30	?	..	Found dead in basement of his home with empty 1 ounce bottle
14	S	M	28	30	30 min.	+	Drank 1 ounce bottle; collapsed, but was able to tell parents what he had done; was driven 20 miles (32 kilometers) in an open car to a police station; died shortly after arrival
15	?	M	32	?	30 min.	+	Found lying beside a liquor bottle and a bottle of "mixer"; vomited and lived approximately thirty minutes before being discovered; nicotine was demonstrated in the vomitus on the floor and in the bottle of "mixer"
16	S	M	32	30	5 min.	..	Found in a state of shock beside an empty 1 ounce bottle; died approximately five minutes later
17	S	F	47	30	5 min.	..	Drank a 1 ounce bottle; died approximately five minutes after taking poison
18	A	M	44	?	10 min.	..	Took "one swallow" of nicotine insecticide, mistaking it for liquor; dead within ten minutes
19	S	M	52	30	10 min.	..	Drank contents of a 1 ounce bottle following a domestic argument
20	S	M	53	30	?	..	Found in a gas-filled room with empty 1 ounce bottle beside him; the body was still warm and artificial respiration was applied "for some time" without success
21	S	M	40	30	?	+	Found dead in hotel room beside an empty 1 ounce bottle; large amounts of nicotine recovered from the stomach content and tissues
22	?	F	53	?	15 min.	+	Given emergency treatment at home for supposed cardiac failure with pulmonary edema; died en route to hospital; one hour after clinical death, muscles of thigh twitched spontaneously; remark by husband that she had been poisoned resulted in an autopsy; large amounts of nicotine recovered from stomach contents and tissues
23	S	M	70	?	30 min.	+	Found in a burning, previously ransacked house; wife's head bashed in with a poker; while being dragged from house, vomited on a police officer's shirt; died about half an hour later; vomitus contained a strong solution of nicotine sulfate; stomach and kidneys contained large amounts of nicotine
24	S	M	35	?	7 hr.	+	A known morphine addict had threatened to commit suicide if returned to jail; while he was in custody, what was thought to be an opium coma developed, the man dying after seven hours; large amounts of nicotine were recovered from the stomach contents, kidney and fragment of liver; source of poisoning thought to have been a tobacco infusion; no other alkaloids were detected in the tissues

* A signifies accidental; S, suicidal

‡ The fatal period in all instances is approximate.

† The "1 ounce bottle" refers to the 1 ounce commercial preparation Black Leaf 40.

the odor of nicotine insecticide by the coroner's deputies left little question as to the manner of death.

The essential findings in the eight cases observed at autopsy were of two types: (1) those showing hemorrhagic gastritis, with or without the odor of nicotine, and often a brownish discoloration of the gastric mucosa, giving the picture of gastritis following the ingestion of a mild caustic; (2) those without gastritis, the findings being essentially those of acute failure of the right side of the heart without an adequate anatomic basis for death. The lungs showed changes commensurate with moderate asphyxia. An almost constant finding was marked hyperemia of the kidneys entirely out of proportion to the acute passive hyperemia elsewhere. Brownish frothy material, not unlike that found in congestive pulmonary edema, was noted about the face and nostrils of the body prior to embalming.

When a history was obtainable, it seemed that collapse occurred shortly after ingestion of the poison, death being deferred for a period of from five to thirty minutes; in one instance (case 22) the clinical symptoms were those of acute cardiac failure. Convulsive phenomena and a typical posture (as is seen in the frog) were conspicuously absent. Crabtree [9] stated that the bodies observed soon after death exhibited unusual hyperthermia.

Pharmacologically, nicotine acts as a stimulant for a short period of time; then depression or paralysis develops. Centrally, on the medullary centers there is respiratory stimulation; in toxic doses, the poison produces convulsions of the medullary type. Salivation, emesis and fibrillary contraction of skeletal muscle are due to its central action. Sollmann [10] stated that death is due to medullary paralysis. Nicotine also has a curare-like action. Peripherally it first stimulates, then paralyzes all the autonomic ganglions. Franke and Thomas [4] felt that death is due to a like effect on the respiratory muscles, peripheral paralysis occurring if convulsions are prevented, and fixation of the respiratory muscles, if convulsions occur. They feel that the reflex paralysis is peripheral rather than central and that nicotine poisoning is an acute emergency doing no irreparable damage. Moore and Row [11] suggested that the effect of nicotine could be due to this curare-like property as readily as to its central action. Gold and Brown [12] cited evidence that nicotine produces peripheral respiratory paralysis.

9. Crabtree, R. L. (chief deputy coroner of Multnomah County): Personal communication to the authors.

10. Sollmann, T.: Manual of Pharmacology, Philadelphia, W. B. Saunders Company, 1932, p. 413.

11. Moore, B., and Row, R.: J. Physiol. **22**:273, 1897.

12. Gold, H., and Brown, F.: J. Pharmacol. & Exper. Therap. **54**:143, 1935.

EXPERIMENTAL OBSERVATIONS

In an attempt to determine the variant effects of nicotine and Black Leaf 40, cavvies were utilized. Two were given subcutaneous injections of 40 per cent aqueous nicotine alkaloid and of Black Leaf 40, respectively, while two others received intragastric injections of similar preparations. The animals receiving Black Leaf 40 exhibited a longer latent period before convulsions started, and the convulsions were of much lesser intensity than those in animals receiving 40 per cent nicotine alkaloid. All died with fixation of the respiratory muscles, the heart continuing to beat. With nicotine alkaloid there was no macroscopic gastritis; microscopically, superficial coagulation necrosis was found. With Black Leaf 40 intense gastritis was manifest, and sections revealed much deeper coagulative necrosis with slight diffuse diapedetic hemorrhage. Evidences of marked renal hyperemia, so consistently seen in cases in man, were absent. Each of the animals presented a moderately dilated right ventricle and atrium, with intense acute passive hyperemia. A dilute solution of sodium hydroxide adjusted to the total alkalinity of the Black Leaf 40 sample failed to produce the same marked inflammatory gastric changes. An attempt to isolate an irritant resin from Black Leaf 40 was unsuccessful.

We wish to emphasize the fact that concentrated solutions of commercial nicotine sulfate (Black Leaf 40) are caustic in action in addition to being toxic, because of their alkaloidal content. The degree of the inflammation produced in the stomach is not necessarily correlated with the concentration of the material there. In our experience, solutions of nicotine, unless alkaline or deteriorated from long standing, do not possess the generally described odor of tobacco, but evoke an irritant acrid postnasal sensation; this may be absent, even in the presence of large amounts of the poison.

Because of the violent toxic action and ready accessibility of nicotine, we feel that commercial preparations of the sort named should be subject to the statutes governing the sale of poisons and their sale by nonpharmaceutical dealers prohibited.

SUMMARY

Twenty-four fatal cases of nicotine poisoning due to drinking insecticides are presented. Black Leaf 40, a commercial insecticide containing nicotine, produces hemorrhagic gastritis. Unusual hyperemia of the kidneys or the presence of a brownish froth about the mouth and nose should suggest nicotine poisoning. Such poisoning, even when the drug has been taken in large amounts, is not necessarily associated with gastritis or with an odor of nicotine. The local irritant action of Black Leaf 40 is not due to its nicotine content.

EPITHELIAL METAPLASIA

AN EXPERIMENTAL STUDY

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Metaplastic phenomena are frequently observed in pathologic material and are a matter of considerable interest. As a rule, the changes are associated with inflammatory or mechanical irritation, but even under such conditions their occurrence is entirely unpredictable. Since experimental work has established two definite morbid states which precipitate epithelial metaplasia in animals, namely, deficiency of vitamin A and stimulation by the ovarian follicular hormone, theelin, it seems worth while to study these factors in the light of their possible relation to the spontaneous lesions seen in human material. It was our aim in the present work to do this by a study of the relative influence of vitamin A deficiency, theelin and chronic irritation.

Information concerning metaplasia is of two kinds. The earliest reports consist of case records and theorization concerning pathogenesis. The substance of these studies is well reported by Borst.[1] The case reports indicate that the usual forms of epithelial metaplasia, if the anaplastic types seen in carcinoma are disregarded, are associated with chronic mechanical or inflammatory irritation. Certain inflammatory lesions seem especially to predispose toward metaplasia—for example, influenza and tuberculous sinuses. The condition is not infrequent in the renal pelvis, associated with hydronephrosis or calculus.

The newer knowledge of metaplasia is the product of experimental studies. These have shown two dependable mechanisms by which metaplasia of various epithelial surfaces may be produced, namely, by feeding a diet deficient in vitamin A and by overdosing with theelin. The studies of vitamin A deficiency have become more numerous in recent years. The conditions under which lesions occur and the character of the lesions have been fully described by Eddy and Dalldorf.[2] The

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1. Borst, M., in Aschoff, L.: Pathologische Anatomie, ed. 8, Jena, Gustav Fischer, 1936.

2. Eddy, W. H., and Dalldorf, G.: The Avitaminoses, Baltimore, Williams & Wilkins Company, 1937.

transformation affects most epithelial surfaces, commences in the basal layer and may lead to obstruction of ducts and similar consequences.

The studies of metaplasia following administration of theelin are still more recent. Unfortunately none of the experimenters have stated the basal diets employed, so that these studies, with the possible exception of the report of Schmidt,[3] may be said to have been unrelated to the effects of vitamin deficiency. Schmidt studied both the metaplasia produced by vitamin A deficiency and that produced by theelin and also the effect of silver nitrate on epithelium affected by both conditions. His results are referred to in more detail later on.

The net results of these various studies have been divergent, and our own efforts were planned to correlate the several factors involved. This we have sought to do by control of the diet as to its vitamin A value, while the epithelial cells were simultaneously affected either by theelin or by mechanical irritation.

IRRITATION, VITAMIN A DEFICIENCY AND METAPLASIA

In a group of eight white rats a silk thread was passed through the ventral surface of the trachea and tied on the outside. The skin was carefully sutured to eliminate formation of a sinus, and the animal was fed a diet deficient in vitamin A. Controls were a group of animals with a thread in the trachea and on an adequate diet and a group on a deficient diet but without a thread in the trachea.

The results of this experiment are shown in table 1. In brief, the experiment demonstrates that the thread induced regeneration of the tracheal epithelium, but that the regenerate cells were of their usual character or of the undifferentiated basal type unless the diet was deficient in vitamin A, in which case metaplastic lesions occurred, by predilection about the thread. These results suggest that the supply of vitamin plays a considerable rôle in determining whether simple regeneration or squamous metaplasia occurs about areas of irritation. They led to the following experiment.

THEELIN, VITAMIN A DEFICIENCY AND METAPLASIA

For this experiment castrated immature female rats were used. The first group were on a deficient diet and received theelin in oil in daily doses of 250 international units each, or 25 micrograms of crystalline theelin. Controls were a group of the same number of rats receiving normal diets and theelin and another group on a deficient diet and with-

3. Schmidt, W.: Beitr. z. path. Anat. u. z. allg. Path. **96**:129, 1935.

TABLE 1.—*Effects of Chronic Irritation and Avitaminosis A*

Animal	Sex	Days After Operation	Days on Diet	Vitamin A in Liver, Units	Observation in Thyroid	Observation in Tracheal Epithelium
			Group 1. Animals on a Deficient Diet, with a Thread in the Trachea			
1	M	17	15	S	Moderate hyperplasia, no metaplasia	Regeneration
2	F	28	26	?	Inactive; no metaplasia	Regeneration
3	F	33	31	0	Moderate hyperplasia, no metaplasia	Regeneration
4	M	45	43	0	Slight hyperplasia, no metaplasia	Regeneration
5	F	66	64	0	Moderate hyperplasia, metaplasia	Metaplasia about thread
6	M	81	80	0	Slight hyperplasia, no metaplasia	Metaplasia
7	F	92	91	0	Inactive; no metaplasia	Metaplasia
8	F	97	96	0	Moderate hyperplasia, no metaplasia	Metaplasia
			Group 2. Animals on a Deficient Diet, with No Thread in the Trachea (Controls)			
9	F	..	15	8	Moderate hyperplasia, no metaplasia	No change
10	M	..	29	0	Moderate hyperplasia, metaplasia	No change
11	M	..	43	0	Moderate hyperplasia, no metaplasia	No change
12	M	..	64	0	Moderate hyperplasia, metaplasia	No change
13	F	..	78	0	Moderate hyperplasia, no metaplasia	No change
14	M	..	97	0	Moderate hyperplasia, metaplasia	No change
15	M	..	114	0	Moderate hyperplasia, no metaplasia	Metaplasia in one patch
16	F	..	125	0	Moderate hyperplasia, metaplasia	Metaplasia, few patches
17	F	..	135	0	Slight hyperplasia, no metaplasia	Metaplasia, few patches
			Group 3. Animals on a Normal Diet, with a Thread in the Trachea (Controls)			
18	F	15	..	100	Epithelial activity, no metaplasia	Necrosis
19	M	18	..	200		Desquamation
20	M	31	..	200		Regeneration
21	M	34	..	300		Regeneration
22	F	41	..	300		Regeneration
23	F	52	..	400		Slight thickening
24	F	64	..	300		Regeneration and flat surface cells
25	F	67	..	300		Necrosis
26	M	69	..	300		Normal type, with inflammation
27	M	82	..	1,000		Regeneration and flat surface cells
28	M	91	..	800		Epithelium absent at site of thread, elsewhere normal type
29	M	104	..	1,600		Normal type

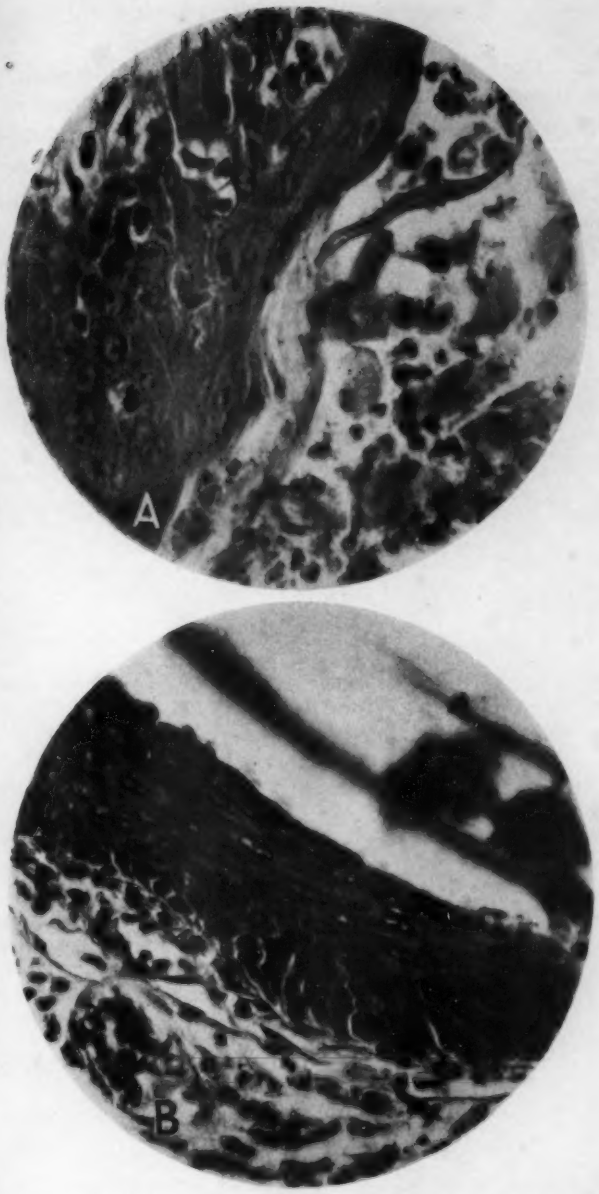

Fig. 1.—The varied response of tracheal epithelium to a foreign body (thread) when the diet is deficient, and when it is adequate, in vitamin A: *A*, epithelium of a rat (table 1, no. 5) which had a thread in the trachea and which had been on a deficient diet for sixty-four days. The epithelium in contact with the thread is hyperplastic and metaplastic; the surface is replaced by non-nucleated squamous cells. Note the desquamated keratinized plate. *B*, tracheal epithelium of a rat (table 1, no. 27) which had a similar foreign body for eighty-two days but was on a normal diet. This epithelium is also hyperplastic and has been flattened, but no keratin is present, and all of the cells are nucleated. The indistinct mass near the surface is part of the thread.

out theelin. The results of this study are shown in table 2. It may be seen from these results that metaplasia regularly occurred in the endometrium if theelin was given and the diet was deficient, while the control groups did in no case show metaplasia, presumably, in the one case, because the period of deficient diet was too short and, in the other, because the intake of vitamin A prevented metaplasia.

The fourth animal in group 1 received identical treatment until fourteen days before the end of the experiment, when it was fed large amounts of cod liver oil. Despite the continued administration of

TABLE 2.—*Effects of Theelin and Avitaminosis A*

Animal	Days After Operation	Days on Diet	Days on Theelin	Vitamin A in Liver, Units	Appearance of Cervix and Vagina	Metaplasia in Endometrium
Group 1. Oophorectomized Animals Given a Deficient Diet and Theelin (Rat 4 Had, in Addition, Cod Liver Oil for Fourteen Days Before the End of the Experiment)						
1	34	34	29	0	Estrus	Present
2	44	44	39	0	Estrus	Present
3	63	63	58	0	Estrus	Present
4	70	56	66	2,000	Estrus	Present; beginning repair
Group 2. Oophorectomized Animals Given a Normal Diet and Theelin (Controls)						
5	34	34	29	Present	Estrus	None
6	46	46	41	300	Estrus	None
7	61	61	56	800	Estrus	None
8	72	72	67	2,000	Estrus	None, proliferation of basal cells
Group 3. Oophorectomized Animals Given a Deficient Diet, with No Theelin (Controls)						
9	32	32	..	0	Anestrus	None
10	45	45	..	0	Anestrus	None
11	60	60	..	0	Anestrus	None
12	62	62	..	0	Anestrus	None

theelin, this animal showed metaplasia undergoing repair, conforming precisely to the mechanism of repair described by Wolbach.[4]

METAPLASIA OF THE THYROID GLAND AND VITAMIN A DEFICIENCY

An interesting incidental observation in the first group of animals is also recorded in table 1, namely, that minute metaplastic lesions are frequent in the thyroid glands of rats long maintained on a diet deficient in vitamin A and that the lesions are regularly associated with hyperplasia of the glandular epithelium. Regeneration and metaplasia occurred together in vitamin A deficiency. Regeneration, but no metaplasia, occurred in the controls on the stock diet.

4. Wolbach, S. B.: J. Exper. Med. **51**:511, 1933.

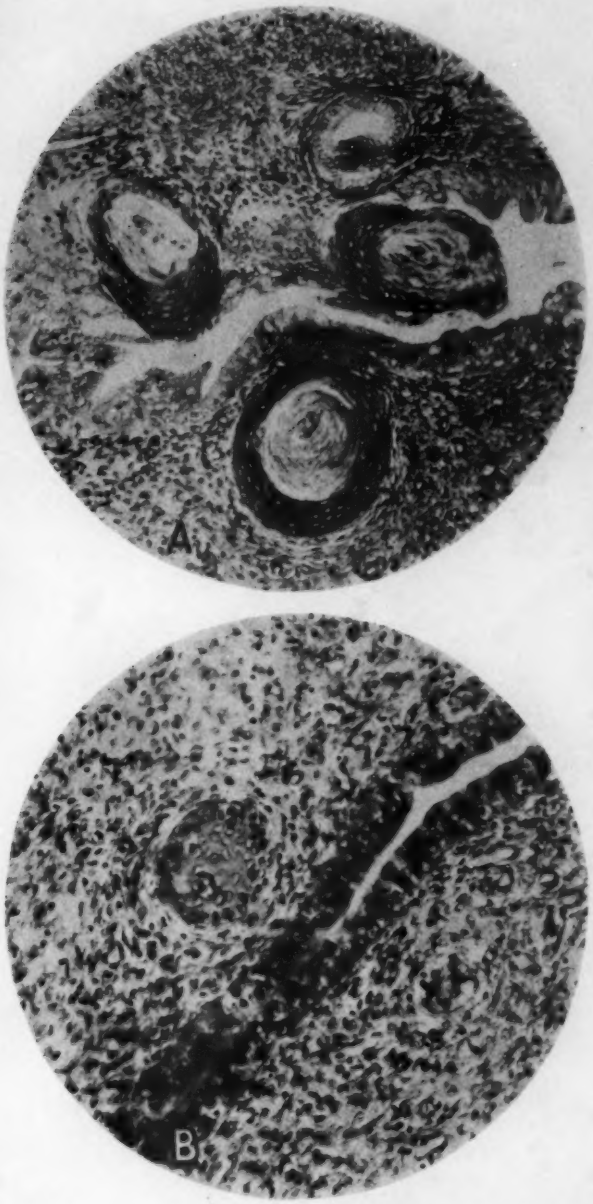

Fig. 2.—The varied response of endometrial epithelium to stimulation by theelin, depending on the supply of vitamin A. *A*, a thick hyperplastic epithelium and three foci of squamous metaplasia, two occupying the mouths of glands (rat 3, table 2). *B*, epithelium from a rat (table 2, no. 7) treated similarly but given adequate vitamin A. This epithelium is also in the estral phase, is thick and hyperplastic, but shows no areas of metaplasia.

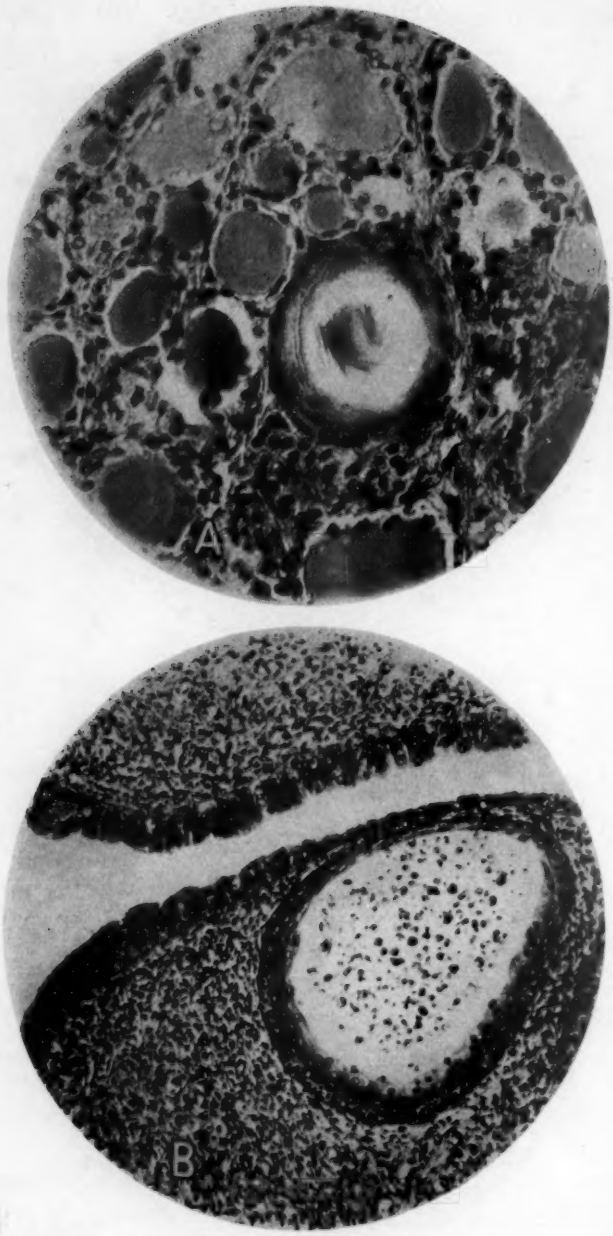

Fig. 3.—*A* shows a metaplastic focus in the thyroid gland after vitamin A deficiency (rat 10, table 1). The central mass consists of a plug of non-nucleated, keratinized scales surrounded by squamous epithelium. *B* shows repair after metaplasia in a rat (table 2, no. 12) which had been on a deficient diet and theelin for approximately two months and which was then given cod liver oil. The theelin was continued. Notice the activity of the basal cell layer. The keratinized surface has desquamated, and keratinized fragments lie within the gland.

COMMENT

In these studies we have taken as criteria of genuine metaplasia the appearance of true keratinization, the formation of flat, non-nucleated surface cells and the presence of keratohyaline granules and intercellular bridges. If these standards are accepted, the results indicate that the factor indispensable to metaplasia in the experiments described was the dietary deficiency of vitamin A. In no case was genuine metaplasia produced when this factor had been fed to the animal or when the animal's liver was found to contain appreciable amounts of the vitamin.

The action of theelin, as well as that of mechanical irritants, appears to have been a stimulant to growth, and each of these factors was capable of producing metaplastic changes in the epithelia affected, but only if the diet was deficient in vitamin A. It seems apparent from an examination of the records that it was increased rate of growth, due in the case of the thread to increased destruction of the tracheal cells, which precipitated what we believe to be a premature metaplasia due to deficiency of vitamin A. This view is supported by the following facts: first, that metaplasia did not occur in the presence of stores of vitamin A in the liver or of adequate intake of the vitamin in control experiments and, second, that the site of epithelial regeneration in each case proved to be the site of metaplastic degeneration. The evidence on this point is further supported by the occurrence of metaplasia associated with hyperplasia in the thyroid and the behavior of animal 4 (table 2), in which repair of well advanced metaplasia was induced by two weeks' feeding of cod liver oil, despite the continuance of the administration of theelin.

If these facts are now followed to their logical conclusion, it appears that, of the various factors studied, vitamin A deficiency alone appears capable of producing metaplasia in established epithelia but that such changes may be prematurely precipitated by secondary factors which increase the rate of growth of the cells. This is in harmony with the generally accepted point of view, first propounded by Wolbach and Howe,[5] that vitamin A is essential to the growth of certain epithelial cells.

These results agree with those of Schmidt,[3] who was able to produce abnormal keratinization of the vaginal epithelium of the rat by means of both vitamin A deficiency and theelin and who hastened the lesions in both instances by applications of silver nitrate. Other work with the hormone cannot be interpreted in the light of our own experience, since the dietary is in no case known. The repeated observation that a very early effect of vitamin A deficiency is a prolongation of the

5. Wolbach, S. B., and Howe, P. R.: J. Exper. Med. **42**:753, 1925.

stage of keratinization during estrus naturally accords well with our own experience, since these results have occurred in animals subject to the stimulation of follicular hormone.

Other lesions of vitamin A deficiency, while they follow a fairly regular order of organs involved, are unpredictable as to the precise sites in the organs in which the nests of metaplastic cells will develop. It seems probable that the order of organs involved is dependent on the rate of growth of the cells of those tissues and that the irregular appearance of metaplasia in a single structure is due to the lack of precipitating factors.

Evidence of vitamin A deficiency of a functional rather than structural nature is also exaggerated by excessive requirements. In the retina, where the vitamin plays an important part in the formation of visual purple and in the perception of light, exposure to strong light, if the person is moderately deficient, intensifies the functional deficiency of the vitamin.[2]

When we turn to the spontaneous metaplastic lesions seen in man we find that factors which destroy epithelial cells are usually present. These most frequently are chronic inflammation and prolonged mechanical irritation. Of the latter, the indwelling cannula is sometimes responsible for metaplastic changes in the epithelium it lies against, or a calculus in the pelvis of the kidney may be responsible. We have studied a number of cases of metaplasia, which is not infrequent, for instance, in infants suffering from pulmonary diseases. Several examples have been found in this laboratory. We have also seen two examples of extensive metaplasia of the renal pelvis. Metaplasia is seen in chronic atrophic rhinitis, in which it is associated with prolonged inflammatory changes. It is seen in cervical polypi, usually on exposed surfaces. In the respiratory tract of the adult lesions are most frequent near the bifurcation of the trachea, especially adjacent to tuberculous lymph nodes. It also regularly occurs in human beings suffering from vitamin A deficiency, as many reports have shown.

The question naturally arises whether the spontaneous lesions in man are primarily due to an underlying, if partial, vitamin A deficiency. Our present results suggest that this is true. Other facts which support this view are, first, that the incidence is highest in patients with chronic debilitating illnesses, in which deficiency phenomena are common; second, similar lesions are common in patients with biliary atresia, in which vitamin deficiency is known to exist, and, finally, the lesions are identical with those produced by vitamin A deficiency.

In several recent cases of metaplasia studied in this laboratory, including one in which atrophic rhinitis and one in which renal metaplasia was present, a search was made for confirmatory evidence of vitamin A deficiency, using the photometer test, with examination of

the skin for signs of keratosis, the corneal surfaces for dryness, and so on. In these cases nothing of importance was found. However, both cases came to our attention after the patients had had some weeks of hospital care, and it is true that all gave a history of meager diets.

It is possible that foci of spontaneous metaplasia may develop in persons whose vitamin A deficiency is relative and slight under the stimulus of secondary factors inciting epithelial growth. This would be in accord with a variety of other observations concerning the "subclinical" stages of the deficiency disease. Careful clinical studies may contribute valuable information on this point, and measurements of the serum concentration of the vitamin at the earliest opportunity may prove helpful.

The observations of the thyroid are in accord with other work. The gland becomes hyperplastic in vitamin A deficiency, as Wendt and others have shown. Under these circumstances metaplasia may be expected to occur early.

SUMMARY

In a number of experimental studies of the effects of theelin, mechanical irritation and vitamin A deficiency on epithelial metaplasia it appeared that vitamin A deficiency is the primary, essential condition for metaplasia and that theelin and mechanical irritation act solely as secondary factors. Metaplasia commonly develops in the thyroid gland during vitamin A deficiency but only in association with epithelial hyperplasia.

APPENDIX

Basal Diet.—The basal diet used was deficient in vitamin A and was a modification of Sherman's test diet 379. Its composition was as follows:

	Percentage
Casein, previously extracted with hot alcohol	18
Starch	65
Dried brewers' yeast	8
Hydrogenated cottonseed oil	5
Osborne-Mendel salt mixture	4

The components of the diet were well mixed. Each animal received all it would eat daily.

Stock Diet.—The normal diet contained whole oats, white bread, lettuce and carrots plus cod liver oil (1 cc. every second day).

Vitamin A Assay.—Tests for vitamin A (Carr-Price reaction) were made on each lot of the basal diet and on the liver of each animal. The method was the routine procedure for assay of vitamin A in this laboratory and consisted in making an extract of the material with purified petroleum benzine (petroleum ether), evaporating it and dissolving the residue in chloroform. Suitable amounts were evaporated to 0.2 cc. and treated with 1 drop of acetic anhydride and 1 cc. of

antimony trichloride in chloroform. The blue color was compared with a solution of copper sulfate and ammonia,[6] to estimate its strength in Lovibond units. The extract of the basal diet was made directly by adding purified petroleum benzine. The livers were first ground with sand and 95 per cent alcohol and centrifugated. The supernatant liquid was extracted with purified petroleum benzine.

Histologic Technic.—The trachea, vagina and uterine horn were fixed in Zenker's fluid. Median sagittal cuts were made of the vagina. The trachea was split lengthwise on the posterior surface and pinned flat on cork. The tissues were dehydrated in these positions and embedded in paraffin. Sagittal sections were made of the vagina. Plane sections were cut through the uterine horn. Serial sections of the trachea were made parallel to the long axis. Staining was by the hematoxylin-eosin method and by Unna's method for keratohyalin.

6. Menken, J. G.: Deutsche med. Wchnschr. **58**:1484, 1932.

Case Reports

DIFFUSE MESOTHELIOMA OF THE LEPTOMENINGES ASSOCIATED WITH CORTICAL AND VENTRICULAR GLIAL HERNIAS

A Clinical-Pathologic Study and Report of a Case

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CHICAGO

A primary diffuse tumor of the leptomeninges is rare when compared with the metastatic invasion of the subarachnoid space by tumor cells arising elsewhere in the body. The metastatic lesion has been properly designated as diffuse leptomeningeal carcinomatosis, sarcomatosis or gliomatosis, depending on the histologic nature of the parent neoplasm. Primary diffuse leptomeningeal tumors present varying histologic pictures and have been described as meningeal sarcoma, endothelioma, perithelioma, etc. The case described here is one of a primary diffuse tumor which originated from the "lining cells" of the leptomeninges.

REPORT OF CASE

A white boy aged 15 months, with the diagnosis of tuberculous leptomeningitis, was admitted to the Cook County Hospital on July 6, 1936. The mother stated that the child had been apparently well until two weeks before admission, when she noticed that his right eye was turned inward. At the same time he began to vomit about fifteen minutes after eating, three or four times a day. As the child was failing rapidly, the mother was advised by her physician to bring him to the hospital.

The birth, the feeding and the developmental and family histories showed nothing significant.

The child was poorly nourished; the temperature was 98.6 F., the pulse rate 124 and the respiration rate 24. The throat was slightly injected. The chest and abdomen revealed no noteworthy findings. There was bilateral strabismus. The abdominal reflexes were not elicited; the Brudzinski sign was positive.

The tentative diagnosis was tuberculous leptomeningitis. The spinal fluid, which was clear, was under increased pressure. There were 3 cells per cubic millimeter; the Pandy reaction was 1 plus; no pellicle formed when the fluid stood

From the Pathologic Laboratories (Dr. R. H. Jaffé, director), Cook County Hospital, and the Department of Neurology and Neurosurgery, University of Illinois College of Medicine.

over night, and a smear of a centrifugated specimen was negative for tubercle bacilli. On the following day the left ear drum appeared lusterless, and on the next day, July 8, both ear drums were incised. A considerable amount of pus was obtained, and though the ears drained profusely the child remained listless.

Spinal puncture on July 9 showed clear fluid with 26 lymphocytes per cubic millimeter. The sugar content was 81 mg. and the chloride content 640 mg. per

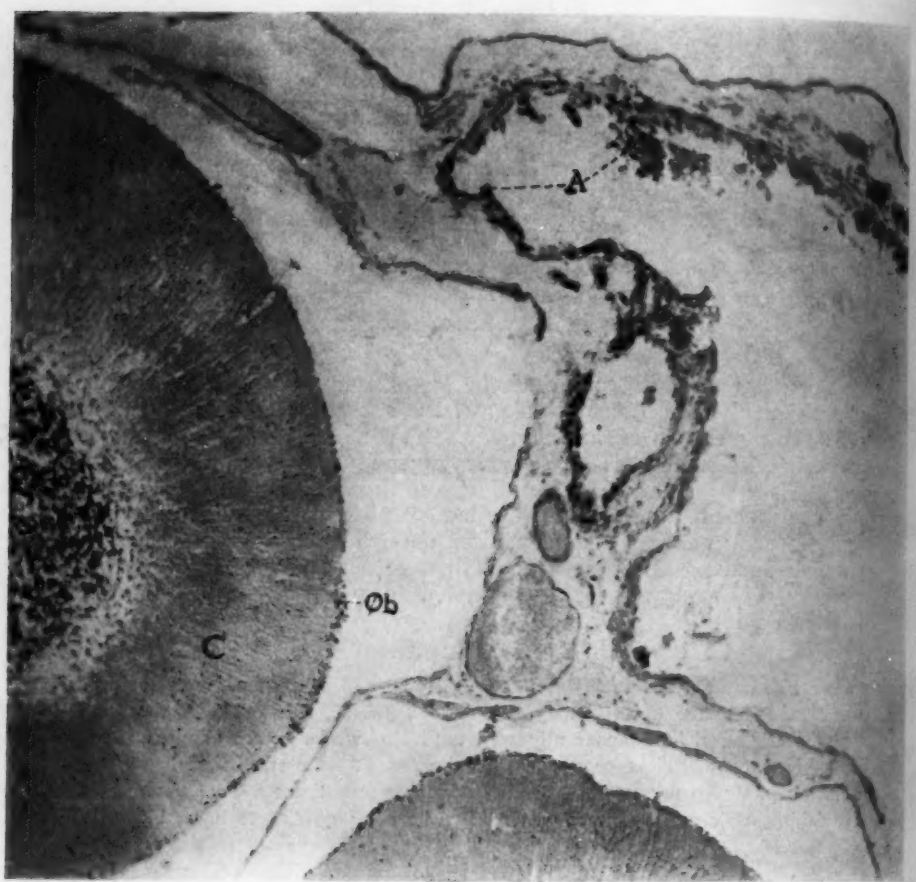

Fig. 1.—Low power photomicrograph of the cerebellum and its overlying leptomeninges. The arachnoid (*A*) shows proliferation of its lining cells. *C* indicates the cerebellum; *Ob.*, Obersteiner's layer. Van Gieson stain; × 100.

hundred cubic centimeters of spinal fluid. The Wassermann reaction of the fluid was negative.

On July 20, the head was found to be enlarged, and a definitely positive Macewen sign was elicited. The pupils did not react to light, and the fundi showed advanced primary optic atrophy. There was no ptosis or strabismus, but the eyeballs failed to move in any direction. There was no facial asymmetry, and the

remainder of the cranial nerves were normal. The child moved all the extremities freely. The muscle tonus in the right leg seemed increased. The achilles tendon reflexes were present bilaterally, but the left patellar and the abdominal reflexes were absent. The Kernig sign was absent, but the Brudzinski sign was definitely present.

The provisional diagnosis was encephalitis, but a congenital intracranial tumor associated with internal hydrocephalus was strongly considered.

A roentgenogram of the skull on July 23 showed a marked separation of all the cranial bones with an increase in the convolutional markings. The sella turcica

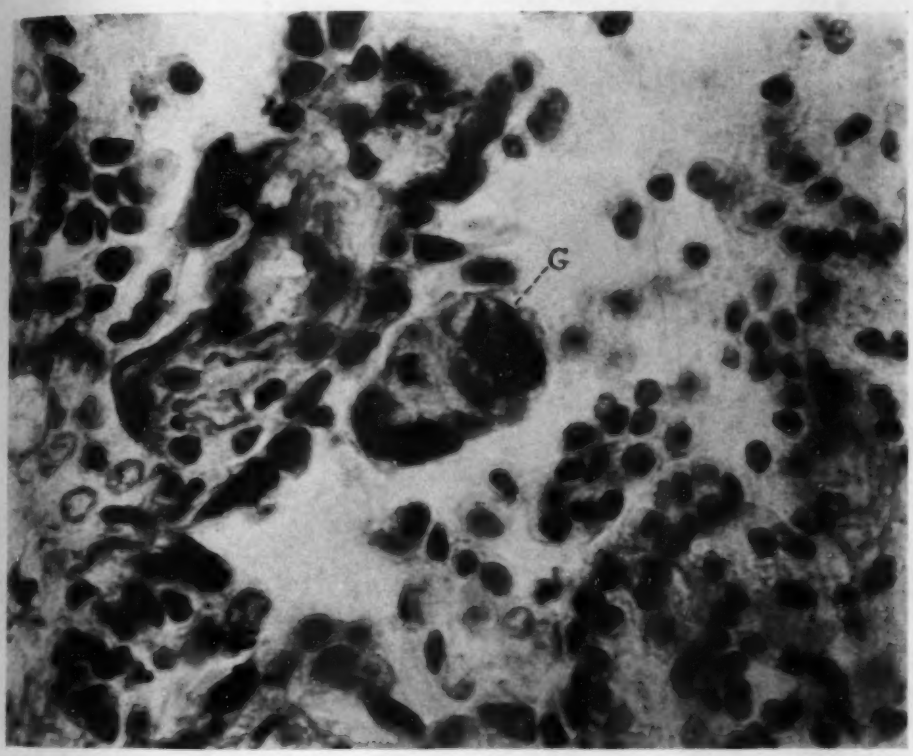

Fig. 2.—High power photomicrograph showing the swollen and proliferated lining cells of the arachnoid membrane; Van Gieson stain, × 600. *G* indicates multinucleated giant cell.

was normal, but there was an area suggestive of suprasellar calcification. On the basis of these roentgenographic findings a diagnosis of suprasellar cyst was also considered.

The red cell count was 7,660,000; the white cell count, 10,850 and later 19,400; the differential count was 58 polymorphonuclear leukocytes, 33 lymphocytes and 9 monocytes.

The child's condition grew steadily worse; dyspnea set in, followed by death on July 25, after a nineteen days' stay in the hospital.

Necropsy.—The postmortem examination was made on the day of death. The head was enlarged, and the cranial bones were thin and separated. Except for focal and confluent areas of bronchopneumonia in the right upper, the right lower and the left lower pulmonary lobes, the essential pathologic changes were in the brain.

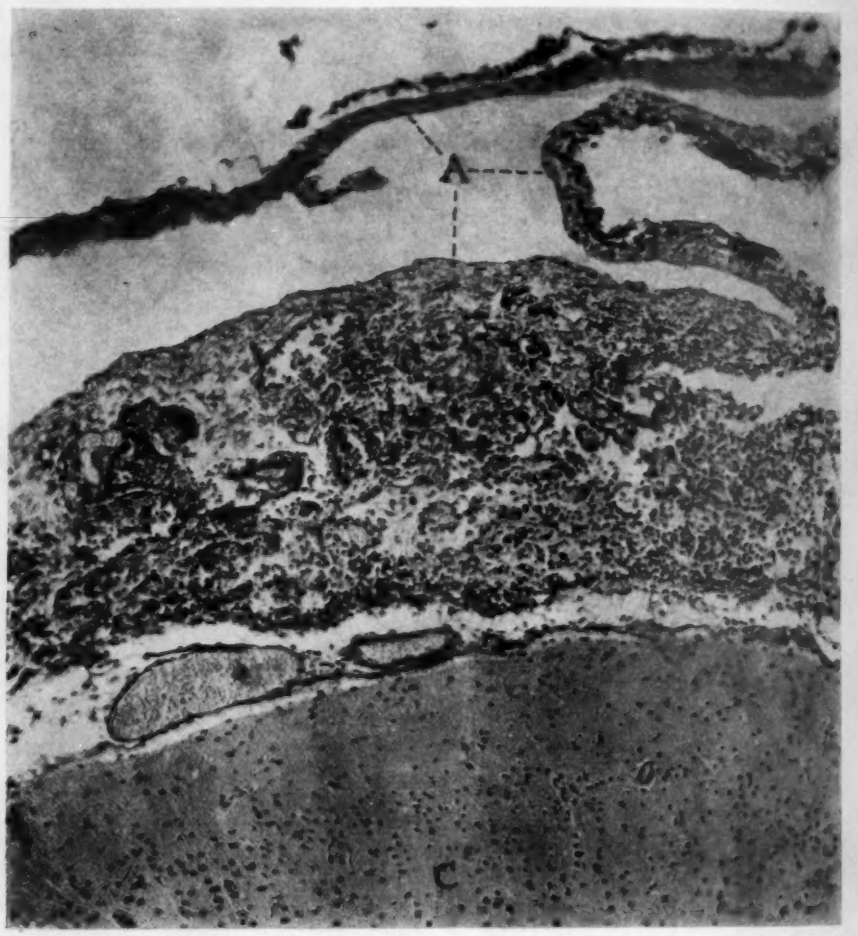

Fig. 3.—Low power photomicrograph showing the very marked thickening of the arachnoid membrane; Van Gieson stain; × 100. *A* indicates the arachnoid; *C*, the cerebral cortex.

The brain weighed 1,200 Gm. The leptomeninges over the convexity were slightly injected, and the convolutions were flattened. Over the base of the brain, in the region of the optic chiasm and the pons, the leptomeninges were thickened and whitish. The infundibulum was bulging and very thin. In the leptomeninges over the base of the brain, especially along the course of the blood vessels, there were innumerable gray-white nodules, varying in size from that of a pinpoint to

that of a pinhead. On sectioning, all the ventricles were enormously dilated and filled with clear fluid, and the ependyma was smooth; the cerebral cortex and the basal ganglions were greatly reduced in thickness.

The diagnosis from necropsy was tuberculous leptomeningitis with excessive internal hydrocephalus. As there was no evidence of tuberculous infection elsewhere in the body, this diagnosis was kept in reserve.

Blocks of tissue were taken from various portions of the brain and embedded in paraffin. Sections were stained with hemalum-eosin and by the methods of Van Gieson, Mallory (phosphotungstic acid), Bielschowsky and Perdrau. Pyroxylin sections were stained with toluidine blue.

Microscopic Examination.—The essential pathologic changes were confined to the leptomeninges. In focal areas, most marked at the base of the brain, the mesothelial cells were swollen and proliferated. In many places single groups of cells hung like bunches of grapes from the walls of the arachnoid membrane (fig. 1). The cells were large, globular, with an ample amount of clear cytoplasm. Their nuclei were round or oval and rich in fine chromatin granules. In many places giant cells were present in the form of large irregular cytoplasmic masses, containing from 2 to as many as 6 round to oval nuclei rich in chromatin (fig. 2). The nuclei were similar to those in the mononucleated cells and contained no mitotic figures. In general, the histologic picture was the same throughout. In the region of the pons, medulla and hippocampal gyri, as well as in the vicinity of the foramina of Luschka and Magendie, tumor masses considerably larger in size were seen (fig. 3). They consisted of dense interlacing and branching arachnoidal trabeculae, which in some places were so closely packed that the tumor assumed a solid appearance. The arachnoidal trabeculae were greatly thickened because of the swelling and proliferation of their mesothelial cells. In many instances, their walls were covered by enlarged globular cells, several layers thick. The central core of the branching column contained elongated and branching strands of reticular and collagenous connective tissue. The innermost layer of the pia showed a normal amount of collagenous tissue, but the mesothelial cells covering the external layer were enlarged and appeared globular. The pial blood vessels showed no pathologic changes, and their adventitial layers were not hyperplastic. The external coats of the blood vessels as they coursed through the pia were intimately surrounded by layers of the aforementioned swollen and proliferated mesothelial cells, giving a picture of a perithelioma. Nowhere did the proliferated cells extend into the brain substance along the invading branches of the pial blood vessels. Where the cellular masses were very dense, there was an increased number of multinucleated giant cells, intermingled with numerous fibrils of reticulin (Perdrau stain). Though very marked in the cores of the arachnoid trabeculae, fibrils of reticulin were also present among individual mesothelial cells. In focal areas single concentric masses of calcific material (psammoma bodies) were present.

In the region of the hippocampal gyrus, the subarachnoid space was distended and filled with bundles of dense fibrillary tissue (fig. 4). This contained round or oval vesicular nuclei, rich in chromatin. When stained by Mallory's method, the cells exhibited a small amount of pale-staining cytoplasm from which issued innumerable fine neuroglial fibrils. This fibrillary glial tissue assumed the aspect of what is seen in so-called isomorphous gliosis, as observed, for instance, in an old patch of multiple sclerosis. No ganglion cells or nerve fibers were found in such bundles. Traced along the subarachnoid space, the glia bundles could be seen to be in direct communication with the molecular layer of some areas of the cerebral

cortex (fig. 4 *C*). In these areas the molecular layer showed a slightly increased number of glia cells, and the covering pia exhibited defects, through which dense masses of glia fibrils protruded into the subarachnoid space, spreading diffusely in all directions and giving a picture of so-called anisomorphous gliosis. At the site of these defects of the pial membrane, which varied considerably in size, strands of the pial connective tissue intimately intermingled with the bundles of fibrillary glia tissue.

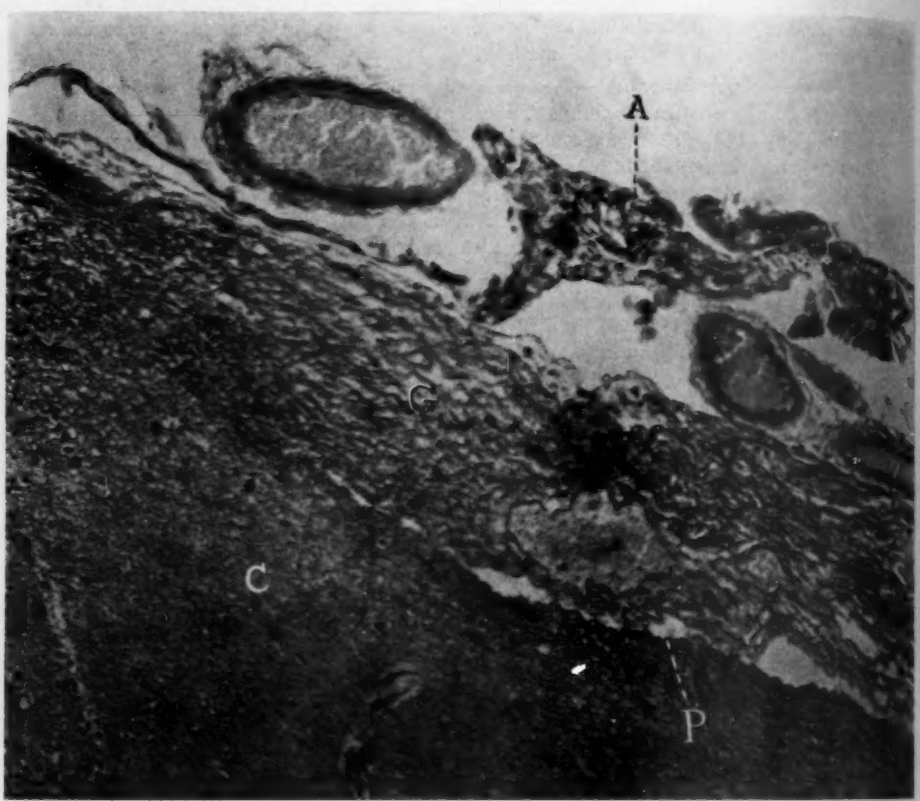

Fig. 4.—Low power photomicrograph showing the origin of the glial bundle (*G*) from the cerebral cortex (*C*) through a defect in the pial membrane (*P*); the arachnoid membrane (*A*) shows proliferation of its covering cells; Mallory's phosphotungstic acid hematoxylin stain; × 150.

Similar glia protrusions or hernias, as they are called, were found in the floor of the fourth ventricle (fig. 5), just medial to the insertion of the tela choroidea (*T*). Here the masses of fibrillary glial tissue which protruded into the fourth ventricle were not covered by an ependymal layer. This tissue consisted of a loose meshwork of fibrillary astrocytes, among which were numerous moderate-sized blood vessels with thickened adventitial layers. By the Perdrau staining method it could be shown that both these ventricular glial masses and the isomorphous gliosis in the subarachnoid space were free from fibrils of reticulin.

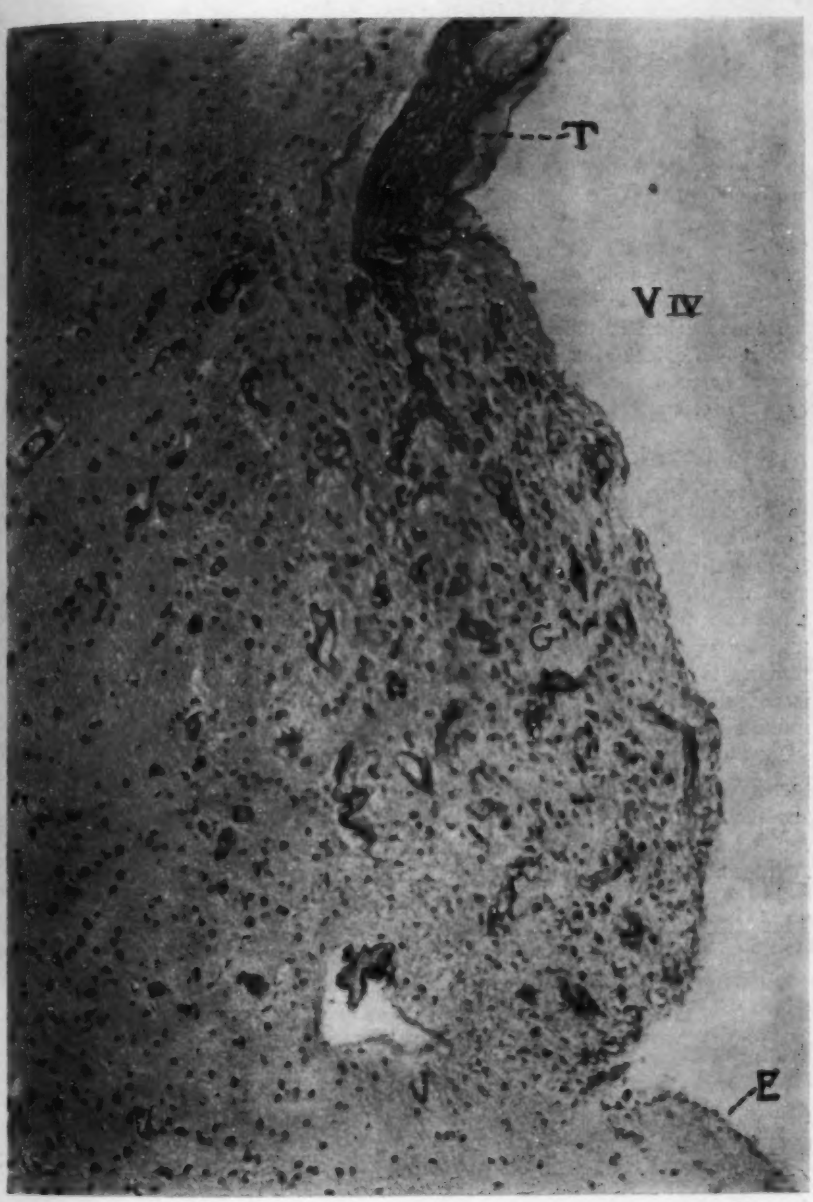

Fig. 5.—Low power photomicrograph of the floor of the fourth ventricle (*Viv*) showing the glial protrusion (*G*) medial to the insertion of the tela choroidea (*T*) lining ependyma (*E*); Van Gieson stain; × 150.

COMMENT

In order to understand better the neoplastic process in our case a brief anatomic description of the leptomeninges may be helpful.

Writing of the leptomeninges, Weed [1] stated:

> The outer layer of these two membranes—the arachnoid—is a cellular structure with a delicate supporting reticulum covered on both sides by characteristic cells. From the inner side of this membrane project small delicate trabeculae upon which are continued the cells forming the inner lining of the arachnoid membrane. The larger of the trabeculae possess a core of a few connective tissue fibrils united on the outside with the fibrous framework of the arachnoid membrane and on the inside with the subpial tissues; the smallest trabeculae seem to be composed merely of the cytoplasmatic processes of the lining cells. The lining cells reflected from the inner surface of the arachnoid membrane and continued upon the trabeculae become the covering cells of the pia mater for this innermost membrane of the central nervous system is formed by this reflection of the lining cells supported by a few connective tissue fibrils and neuroglial elements.

From the histologic study it is apparent that the diffuse tumor in our case was derived from excessive abnormal proliferation of the lining cells of the leptomeninges. In some places the cellular proliferations had intimately surrounded the pial blood vessels, producing a peritheliomatous picture. In histologic appearance this diffuse tumor was somewhat similar to the mesothelial tumors of other serous membranes. That the mesothelial cells of the leptomeninges are capable of the formation of a tumor is evidenced by the occurrence of the meningotheliomatous type of dural meningioma, which most authorities now concede is derived from a proliferation of clusters of so-called arachnoidal cells included within the dura mater.

Considerable controversy has arisen in the literature as to the proper terminology for the leptomeningeal tumors. Oberling [2] noted the similarity of the leptomeningeal cells to the Schwann cells of the peripheral nerves and stated that the leptomeninges were ectodermal in origin. A leptomeningeal tumor derived from the meningoblasts, the parent cells of the arachnoid membrane, was termed by him meningoblastoma. This theory that the leptomeninges are ectodermal was strengthened by the experimental work of Harvey and Burr,[3] who demonstrated the origin of the leptomeninges from the neural crests. These authors proposed the generic term "neurolophoma" for the tumor whose cells of origin are derivatives of the neural crest. In accord with Oberling's proposal, many French investigators speak of the Schwann cells and the leptomeningeal cells as neurogenic derivatives. Thus Cornil [4] and Roussy and Cornil [5] described primary leptomeningeal tumors as gliomas,

1. Weed, L. H.: Bull. Johns Hopkins Hosp. **31**:343, 1920.

2. Oberling, C.: Bull. Assoc. franç. p. l'étude du cancer **11**:366, 1922.

3. Harvey, S. C., and Burr, H. S.: Tr. Am. Neurol. A., 1925, p. 72. Harvey, S. C.; Burr, H. S., and Campenhout, E.: Arch. Neurol. & Psychiat. **29**:683, 1933.

4. Cornil, L.: Bull. Acad. de méd., Paris **110**:307, 1933.

5. Roussy, G., and Cornil, L.: Ann. d'anat. path. **2**:63, 1925.

and Lhermitte and Leroux [6] and Masson [7] classified the neurofibromas of the peripheral nerves as peripheral gliomas.

That the Schwann cells produce reticulin and collagen as do the meningocytes has been shown by Key and Retzius.[8] These cells, therefore, are in no way histologically comparable to the glia of the central nervous system and though of neural crest origin should be spoken of as ectodermal rather than as neurogenic derivatives. A primary glial leptomeningeal tumor has been reported by Roussy, Cornil and Leroux,[9] but recently Bailey [10] considered it a meningioma.

Our case is histologically identical with the case of Eberth [11] in a woman aged 47 and that of Haeger [12] in a male aged 48. As in our case, the cells were mesotheliomatous and intimately surrounded the pial blood vessels, and multinucleated giant cells were present. According to Borst and Ribbert,[13] the presence of such multinucleated cells is characteristic of tumors of the covering cells of "endothelial membranes." As has been noted, the tumor cells were intimately associated with the adventitia of the pial blood vessels. Nowhere was there an increase in the amount of collagenous tissue about the blood vessels. This is a valuable sign, as it helps, in most cases, to distinguish the primary tumor of the leptomeninges from the metastatic invasion of the subarachnoid space by tumor cells, in instances of which there usually is a distinct layer of connective tissue between the blood vessels and the infiltrate. Our case is particularly unusual in that the primary leptomeningeal tumor occurred in a child, for in a child the leptomeningeal tumor is, as a rule, metastatic from a primary tumor elsewhere in the brain, usually a medulloblastoma.

Proliferation of the cells of the arachnoid membrane has been described by Meyer,[14] Cushing and Weed,[15] Essick [16] and others. These clusters of arachnoid cells, like the arachnoid trabeculae, become the seat of calcific deposits with advancing age. Like the mesothelium in our case, these clusters of arachnoidal cells rarely show mitotic figures. A study by Weed has led to the view that calcification represents but one phase of the ultimate fate of these cell clusters; a second, somewhat more striking reaction is represented, not on the side of degeneration

6. Lhermitte and Leroux, R.: Bull. Assoc. franç. p. l'étude du cancer **9**:112, 1920.

7. Masson, P.: Am. J. Path. **8**:367, 1932.

8. Key, E. A., and Retzius, G.: Studien in der Anatomie des Nervensystems und des Bindegewebes, Stockholm, Samson & Wallin, 1875.

9. Roussy, G.; Cornil, L., and Leroux, R.: Rev. neurol. **30**:294, 1923.

10. Bailey, O. T.: Arch. Path. **21**:584, 1936.

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(as in calcification), but on that of outspoken proliferation, which apparently results in neoplasms.

With Weed we designate these lining cells of the leptomeninges mesothelial cells and believe that the term "diffuse mesothelioma of the leptomeninges" is the proper designation for a tumor such as that described. The absence of mitotic figures and of invasion precludes the use of the term "meningoblastoma." Neither are the terms "meningeal glioma" or "primary meningeal sarcoma" applicable, for the proliferating cells are not glial nor do they possess the features of a sarcomatous growth.

Of great interest was the diffuse invasion of the subarachnoid space in certain places by dense bundles of fibrillary glial tissue, which superficially at least resembled a primary glial leptomeningeal tumor. Closer examination showed that the glial tissue was in direct continuity with the cerebral tissue at several points. The glial tissue was seen streaming out through defects in the pia, spreading diffusely through the subarachnoid space. The proliferated or hyperplastic glial tissue in the subarachnoid space was usually isomorphous, but at the site of the bridge connecting this meningeal glial overgrowth the glial bundles were irregularly arranged, giving the picture of so-called anisomorphous gliosis. The subjacent molecular layer of the cortex at these foci showed only a moderate increase in the number of gliocytes.

Similar conditions have been found in different pathologic states, and it appears that they may result from a variety of causes. Thus, in the case reported by Globus,[17] in an 11 month old hydrocephalic child, it was believed that the growth of neuroglia in the subarachnoid space was the result of excessive proliferation of the glia in the brain following strangulation of the blood vessels from thickening and cicatrization of the pia arachnoid.

In Oberling's [18] case the meningeal gliosis was associated with gliosis in the region of the quadrigeminal plate, which had occluded the aqueduct of Sylvius and produced a marked internal hydrocephalus. The dense gliosis penetrated the leptomeninges and spread throughout the subarachnoid space.

Wojno [19] recorded cases of leptomeningeal gliosis in which the invasion was believed to be due to defects in the leptomeninges. Jaffé's [20] case was one of porencephaly in which islands of glial tissue in the subarachnoid space, followed in serial sections, were seen to be in direct continuity with the glial lining of the porencephalic cyst. Primary glial growths in the leptomeninges do occur, as illustrated in Bailey's [10] case, in which an astrocytoma was found in the subarachnoid space, which he believed arose from inclusion of neuroglial cell rests.

In our case the glial hernia was found both in the pia and at the junction of the ependymal layer of the floor of the fourth ventricle with the tela choroidea. It is our belief that the pial and ependymal defects were congenital and in the nature of developmental defects.

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SUMMARY

A diffuse mesothelioma of the leptomeninges, multicentric in origin, in a child is reported. The tumor arose from abnormal proliferation of the lining cells of the leptomeninges. Grossly the brain showed changes suggestive of chronic leptomeningitis. On microscopic examination they proved to be neoplastic. Glial hernias through defects in the pia and the ependyma were present, which were attributed to the developmental defects.

Notes and News

Aid for Medical Research.—The Jane Coffin Childs Memorial Fund is to be established at Yale University. The board of scientific advisors consists of Stanhope Bayne-Jones, R. J. Anderson, Ross G. Harrison, Peyton Rous and M. C. Winternitz. The fund, said to be in the neighborhood of $10,000,000, will be devoted primarily to research on cancer.

The John and Mary R. Markle Foundation, of New York, has appropriated to the National Research Council the sum of $100,000 for support of research in endocrinology for a period of three years. The fund will be administered by a committee of the council's Division of Medical Sciences. Walter B. Cannon, Harvard University Medical School, is the chairman of the committee. Grants will be made in support of approved programs of research in endocrinology.

University News, Promotions, Resignations, Appointments, Deaths, etc.—The University of Colorado has conferred the degree of Doctor of Science on Sara Branham, senior bacteriologist in the United States Public Health Service, in recognition of her work in bacteriology in relation to public health.

Simon Flexner, director emeritus of the Rockefeller Institute for Medical Research, has been elected a foreign associate of the Academy of Sciences, Institute of France.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Effects of Thorium Dioxide. A. J. Fleming and W. H. Chase, Surg., Gynec. & Obst. **63**:145, 1936.

Thorium dioxide, now extensively used as a diagnostic aid in cerebral, peripheral and hepatolienography, its used because it produces no immediate deleterious effect in animals and no definite symptoms in man. However, in common with many other radioactive substances, it has not been in use long enough for one to be certain what its prolonged effect on tissues and organs may be. Concerning the radioactive property of thorium there is no question. It has been estimated that its average period of life is about twenty-five thousand million years and that thorium and the products of its disintegration give off from more than four thousand to over twenty-six thousand alpha particles per gram per second. It is theoretically possible, therefore that thorium may produce a tissue response either mechanically or radioactively. The mechanical effect, like that of a foreign body, would be nonspecific and take place within a few weeks or months. Present observations indicate that this effect is slight and often completely absent in reticulo-endothelial tissues. The radioactive effect, on the other hand, would be infinitely progressive. The present investigation was prompted by the fact that to date there exist conflicting opinions concerning the tissue response to thorium. In one of the cases studied a large firm nodule developed over an antecubital area where thorium dioxide had leaked into the tissues during injection into a vein. The mass consisted of a large amount of pale brownish refractive material, lying partly free but mostly within large macrophage cells, and of dense and hyalinized connective tissue. The process had been developing for five months before excision. In the other instance, thorium dioxide was given to a man with advanced carcinoma, five months before death. Autopsy disclosed no gross evidence of thorium in the reticulo-endothelial tissues. In the liver were diffuse, finely granular, pale brown, refractile masses scattered between the columns of hepatic cells, filling many capillary endothelial and Kupffer cells. The masses were much larger and extracellular around the central veins, so that the parenchymal cells were compressed. In the portal spaces the material was surrounded by fibroblasts and mononuclear leukocytes. The fibroblasts appeared relatively mature, and collagen fibrils were abundant. In the spleen and regional lymph nodes the metallic substance lay mostly in large phagocytic cells in the centers of lymph follicles, although some was extracellular. A definite fibroblastic proliferation was lacking.

In order to determine the accuracy of some observers who have stated that thorium dioxide is without radioactivity the authors exposed the dried chemical to an electroscope and proved that it is radioactive. Its radioactivity in tissue was proved when a bit of lead was photographed by exposure to a frozen section of the liver of the second patient.

It is concluded that while it is impossible to distinguish sharply between the tissue changes which are due to thorium as a mechanical irritant and those resulting from the rays emitted from it, it is reasonable to assume that both mechanical and physical properties are concerned in the tissue responses. Of importance is the fact that the proliferative reaction in both cases was greatest in situations where connective tissue is normally abundant. Blood capillary formation was lacking, the fibrous reaction progressing to hyaline degeneration with impaired nutrition.

Warren C. Hunter.

THE PATHOLOGIC PHYSIOLOGY OF STONE IN THE COMMON BILE DUCT. W. WALTERS, Surg., Gynec. & Obst. **63**:417, 1936.

One or more stones may be present in the common bile duct without producing jaundice. They may produce biliary colic or intermittent fever or both, and either one or both of these conditions together may be associated with pylorospasm. An increase in the diameter of the common bile duct is a sign of obstruction, and the obstruction may be attributable to stones or to pancreatic or other obstruction. In many cases, continuance of symptoms of disease of the biliary tract subsequent to cholecystectomy can be attributed to the presence of forgotten stones of the common duct or to persistent inflammation of the pancreas or sphincter of Oddi, any one of which may obstruct the passage of bile into the duodenum. Factors concerned in the appearance of stones in the common duct are their expulsion from the gallbladder and the formation of soft bile pigment stones secondary to persistent obstruction of the common duct in conditions such as pancreatitis and sphincteritis. Studies of the bacterial flora of bile removed from the common duct in cases of disease of the biliary tract disclose in most cases the presence of bacilli of the gram-negative or colon group, streptococci or staphylococci. All of these are present in some cases. Bacterial studies of bile and analysis of removed gallstones are as necessary as similar studies of the urine and urinary calculi, for since urinary infections have been demonstrated to respond to increasing acidification of the urine and to the use of certain substances, such as the arsenical preparations used in the treatment of syphilis, similar response to biliary infections might result, especially if antiseptic radicals were attached to the halogenated phthaleins which are excreted through the liver into the bile.

FROM THE AUTHOR'S SUMMARY. (WARREN C. HUNTER).

ANATOMIC CHANGES IN RABBITS FROM COAL TAR PAINTING. C. H. HU and S. D. WU, Chinese M. J., supp., 1936, p. 73.

In forty rabbits of different sizes painted with gas-works coal tar on one or both ears once or twice weekly and killed from the seventh to the two hundred and first day after the first painting, there were produced, besides the local changes (epithelial hyperplasia, hyperkeratosis, papilloma formation and acute inflammation of the painted ear and the regional lymph nodes): (1) focal or extensive necrosis of the liver leading to fibrosis or cirrhosis (the lesions at first had a peculiar type of distribution; later there was widespread cirrhosis which might resemble either biliary cirrhosis or nodular cirrhosis with or without marked adenoma-like bile duct proliferation); (2) ascites, due partly to the cirrhotic changes in the liver and partly to peritoneal irritation presumably by coal tar; (3) edema of the subcutaneous tissue, usually associated with the ascites due to peritoneal irritation. Among other changes may be mentioned fatty change of the myocardium and kidneys, deposition of hemosiderin in the spleen, gelatinous degeneration of the marrow of the femur and marked emaciation. The size of the spleen showed no significant change.

FROM THE AUTHORS' SUMMARY.

PATHOGENESIS OF ACUTE DIFFUSE NEPHRITIS: EXPERIMENTAL STUDY OF ALLERGIC LOCALIZATION IN THE KIDNEY. C. G. AHLSTRÖM, Acta path. et microbiol. Scandinav., supp. 29, 1936.

Using injections of staphylotoxin in combination with repeated injections of horse serum, Ahlström was able to produce diffuse glomerular lesions in rabbits. The lesions consisted of intracapillary exudation of a finely granular plasmatic or fibrinous material, accumulation of leukocytes and swelling and proliferation of the glomerular endothelium. Sometimes the glomeruli were strikingly congested; at other times there was a reduced blood content owing to intracapillary exudation and endothelial swelling. He believes that the lesions he produced were of allergic origin because they differed distinctly from the nephrotoxic picture produced by staphylotoxin; they were more pronounced in the animals receiving the

larger doses of serum; they were produced with doses of staphylotoxin considerably smaller than the smallest nephrotoxic dose used for the controls, and the toxin-treated animals showed marked sensitiveness to subsequent small doses of serum. In agreement with Fahr, he concludes that the pathogenesis of acute diffuse glomerulonephritis requires interaction of an allergic status with a specific toxic renal factor.

FREDERICK STENN.

Pathologic Anatomy

THE BRAINS OF INFANTS AND CHILDREN IN RELATION TO POSTMORTEM TIME, TOXICITY AND CONVULSIVE STATE. C. R. TUTHILL, Am. J. Path. **12**:269, 1936.

The brains of infants and young children suffering from acute infection, second degree burns, allergy and lead poisoning did not show changes in the ganglion cells that could be attributed to toxicity. The loss of cytoplasm and the density of its stain, the tingeing of the nerve tissue, the hyperchromatosis of the nuclei and the perivascular spaces varied somewhat with age and the fixative but chiefly with the time elapsed post mortem. The indications of toxic injury were found rather in the reactions of the microglia and the macroglia. The most severe and most frequent lesions in the brains were those produced by the vascular disturbances of the convulsive states which accompany many diseases of infancy and early childhood.

FROM THE AUTHOR'S SUMMARY.

AMYLOIDOSIS OF LUNGS AND HEART. H. W. FERRIS, Am. J. Path. **12**:701, 1936.

In most cases of generalized amyloidosis the lung shows scant deposits of amyloid or none; such deposits as occur are noted about blood vessels or within the alveolar walls. In some instances amyloid deposited in large amounts, frequently in nodular form, greatly alters the pulmonary architecture. In most of these cases there is no preceding or concurrent chronic disease usually regarded as the precursor of amyloid. Advanced amyloidosis of the lungs and heart may produce cardiac failure and death.

FROM THE AUTHOR'S CONCLUSIONS.

PARAPHYSIAL CYSTS. A. J. MCLEAN, Arch. Neurol. & Psychiat. **36**:485, 1936.

Two cases of so-called paraphysial cysts, commonly known as cysts or so-called tumors of the third ventricle, are described in detail by McLean. One was in a man aged 23 who died suddenly after nine months of illness; the other was in a girl aged 16, in whom the tumor was diagnosed by ventriculography and successfully removed. The histologic examination of the capsule of the tumor revealed fibrogelatinous connective tissue, rich in capillaries; the inner lining of the capsule was covered with cylindric and cuboid ciliated epithelial cells, many of which contained granules of lipochrome pigment. The contents of the tumor were gelatinous and colloid. McLean's findings sustain Sjövall's observations that the cystic tumor of the third ventricle originates from the vestiges of the paraphysis which exists in the human embryo.

G. B. HASSIN.

THE KLIPPEL-FEIL SYNDROME. L. W. AVERY and C. C. RENTFRO, Arch. Neurol. & Psychiat. **36**:1068, 1936.

This syndrome was observed by Avery and Rentfro in a deformed girl who was born by normal delivery and died thirty-five days later. The upper four cervical vertebrae were absent; the hair line was low, and the head, which had a circumference of 35 cm., rested directly on the thorax. The spinous processes of all the cervical and upper four dorsal vertebrae were absent, and the spinal cord, in the cervical region, was divided into two parts by a deepened sulcus; the anterior commissure was absent, and the posterior surface of the spinal cord had no pia, while that in the lower cervical division of the cord, where it formed two

abnormal fissures, was thickened. In the cervical region there was no central canal, but a double canal was present in the lumbar region. In the thoracic region there was distinct gliosis in the posterior columns. Of the roentgenographic findings, the most important were partial fusion of the second thoracic with the seventh cervical vertebral body and clefts in the occipital bone and in the arches of the first dorsal and all the cervical vertebrae. The authors think that the cause of the anomalies was dysraphia—incomplete closure of the neural plate. The reduction in the number of vertebrae, they think, was secondary.

G. B. HASSIN.

SEVERE MYOCARDITIS OF UNKNOWN ETIOLOGY. H. B. TAUSSIG and E. H. OPPENHEIMER, Bull. Johns Hopkins Hosp. **59**:155, 1936.

A child presented syphilitic infection following an intramuscular injection of whole blood. At first she responded well to treatment, and then, two months later, a cutaneous eruption suddenly developed, and she died of cardiac failure. Autopsy revealed extensive diffuse myocarditis in the form of round cell infiltration and tubercle-like proliferative lesions. There were similar lesions in the lungs, liver and lymph nodes. Even though the clinical events preceding death made syphilis appear to be the most probable cause of the disease, and the pathologic observations were also consistent with severe syphilitic infection, the authors were unable to establish the diagnosis. Consequently, although they believe the disease may have been syphilitic in origin, they think it is entirely possible that it was caused by some other unidentified infectious agent. The case, however, was so remarkable and the lesions so striking that they put it on record in the hope that through further reports of similar cases the nature of the disease may ultimately be recognized with certainty. FROM THE AUTHORS' SUMMARY.

THE PATHOLOGIC CHANGES RESULTING FROM VITAMIN DEFICIENCY. S. B. WOLBACH, J. A. M. A. **108**:7, 1937.

Knowledge of the morphologic consequences of vitamin deficiencies is limited and has not extended beyond obvious tissue and cytologic changes.

The pathology of vitamin A deficiency indicates that the seat of the physiologic disturbances is in the epithelial cells. Chemical rôles are suppressed, but proliferative powers are not inhibited; neither are the potentialities of cells lost, as is shown by the return to normal physiologic function when vitamin A is restored to the animal.

Vitamin C deficiency affects mesenchymal tissues, the most obvious morphologic consequence being the effect on the formation and maintenance of intercellular materials.

Vitamin D deficiency, besides the well known effect on calcium and phosphorus metabolism, suspends the cartilage cell cycle essential to endochondral growth of bone, though not impairing multiplication of the cartilage cells.

Vitamin B_1, as is indicated by phenomena in recovery from the deficiency, must be more directly concerned in the physiology of the nervous system than is any other vitamin. The sudden return to normal function after administration of vitamin B_1, before morphologic repair has been initiated, warrants this conclusion.

A reasonable working hypothesis is that each vitamin is necessary in certain cells for one type of chemical mechanism, the suppression of which is compatible.

THE RÔLE OF PARALLERGIC REACTIONS ON THE NERVOUS SYSTEM. J. M. LASOVSKY and M. M. KOGAN, Arch. internat. de méd. expér. **11**:505, 1936.

The parallergic reaction is defined as the phenomenon of marked sensitization of the tissues of a sensitized organism to nonspecific excitants. After sensitizing rabbits to horse serum, Lasovsky and Kogan subjected the skin of the hindfeet to refrigeration. An inflammatory reaction was observed early in the refrigerated skin and subjacent muscular tissue. The subdermal tissue showed hyperemia,

edema and leukocytic and histiocytic infiltrations; the walls of the small arterioles were necrotic; the muscles and nerve fibers showed degenerative changes, and even a typical Arthus phenomenon was seen. These features were absent in the nonsensitized controls. They contended that the disturbance of nerve fibers is primary and that the nervous system takes an important part in allergic as well as parallergic reactions.

FREDERICK STENN.

PATHOGENESIS OF PLACENTAL OBLITERATION. W. VOLLAND, Virchows Arch. f. path. Anat. **296**:690, 1936.

Thirty full-term normal placentas were subjected to histologic study by means of various staining methods to determine the nature and the development of the obliterated areas of the placenta, usually referred to as white infarcts. The condition is the result of a precipitation within the intervillous spaces of finely fibrillated hyaline fibrinous material. The hyaline material may represent the gel stage of the formation of fibrin. The stroma of the villi in the obliterated areas gives the metachromatic staining reaction with methyl or gentian violet of amyloid. In explanation of the intervillous precipitation or thrombosis Volland accepts the concept of Dietrich, who ascribed intravascular thrombosis to an alteration in the relation of plasma and endothelium to each other, especially in the sense of an activation of the endothelium akin to sensitization. Support of this view in placental obliteration Volland finds in swelling, proliferation, budding and desquamation of the villous syncytium, which is functionally homologous with vascular endothelium, in which Dietrich has described similar changes associated with thrombosis. Volland admits morphologic similarity between the changes observed in placental obliteration and those of the hyperergic tissue reaction and accepts the possibility of an allergic state of the placenta in certain toxicoses, but he is not willing to admit that in every instance placental obliteration is an allergic hyperergic response.

O. T. SCHULTZ.

A CASE OF TRUE HERMAPHRODITISM. S. LINDVALL and F. WAHLGREN, Virchows Arch. f. path. Anat. **297**:1, 1936.

A 19 year old person with predominately male anatomic and psychic characters had a small hypospadiac penis and a descended small right testis. There was no beard, the breasts were like those of a young woman and the pelvis was of female type. Regularly at monthly intervals the urine became bloody. At operation a uterus with a left tube and ovary were removed. The ovary was the size of that of a sexually mature young woman and was functioning actively. A portion of testis removed for biopsy revealed normal seminal tubules but no spermatogenesis; there were very few interstitial cells. The authors consider their case to be one of gynandromorphism, in which the fertilized ovum is bisexual in its chromosomal constitution, rather than one of intersex, in which the developing embryo has the chromosomal constitution of one sex, but later the sexual characters undergo alteration to those of the opposite sex. In the older classification of Klebs the reported case would be one of true lateral hermaphroditism. Medicolegally it would have to be considered an example of sexus anceps, in which the sex cannot be legally determined.

O. T. SCHULTZ.

CHANGES IN THE APPENDIX IN GASTRIC ULCER. P. W. SSIPOWSKY, Virchows Arch. f. path. Anat. **297**: 16, 1936.

Sixteen appendixes obtained in necropsies of persons with gastric ulcers were subjected to histologic study, especial attention being paid to the nerve elements. Various changes, mostly atrophic in character, were noted in the mucosa. The intrinsic nerve fibers were swollen, beaded and sometimes fragmented. Some of the nerve cells were enlarged and had pyknotic nuclei. Similar alterations were observed in a series of control appendixes removed from elderly persons, but they were less constant. In every case the changes noted in the intrinsic nerve elements were similar to those seen in the stomach in the region of the ulcer. Ssipowsky

concludes that the alterations in the nerve elements of the appendix arose at the same time as those in the stomach and had the same underlying cause, namely, a pathologic state of the entire vegetative nervous system. O. T. SCHULTZ.

SIDEROFIBROUS NODULES OF THE STOMACH AND LIVER OF TRAUMATIC ORIGIN. S. MOESCHLIN, Virchows Arch. f. path. Anat. **297**:63, 1936.

Siderofibrous nodules, so-called Gandy-Gamna nodules, composed of connective tissue, foreign body giant cells and iron pigment, occur most often in the spleen. They are held by most writers to be the result of localized hemorrhages; a number of French writers believe them to be due to the presence and growth of molds. The case reported was that of a 2 year old child who had swallowed a saddler's awl. Three weeks later the symptoms indicated that the awl had penetrated the psoas muscle. After a further three weeks the implement was removed surgically; death occurred the following day. In the prepyloric region of the stomach, where the awl had apparently first penetrated the wall of the stomach, were two pea-sized brownish nodules, and in the adjacent adherent liver were others. The nodules had the characteristic histologic structure of fibrosiderous nodules of the spleen. O. T. SCHULTZ.

ENTEROCHROMAFFIN CELLS IN THE BILIARY PASSAGES. V. ERSPAMER, Virchows Arch. f. path. Anat. **297**:70, 1936.

Argentophile chromaffin cells occur normally in the alimentary tract, from the esophagus to the anus, in many species of animals. Erspamer found the cells, under normal conditions, in or just beneath the epithelium of the gallbladder of man, beef, dog, swine and guinea-pig and in the intrahepatic ducts of these and other species except the beef. The cells make their appearance during the second half of fetal life. Their number is increased in chronic inflammations of the gallbladder and biliary passages, especially such as are associated with epithelial hyperplasia. Accepting Masson's view that carcinoids of the alimentary tract are derived from the enterochromaffin cells, Espamer believes that the occurrence of such tumors in the biliary tract is theoretically possible. The function of the cells is unknown. He inclines to the view that they have a part in the intermediary metabolism of phenol compounds. O. T. SCHULTZ.

Microbiology and Parasitology

EXPERIMENTAL TUBERCULOSIS BY INTRACEREBRAL INOCULATION. K. C. SMITHBURN, J. Exper. Med. **64**:771, 1936.

Intracerebral inoculation of tubercle bacilli in normal guinea-pigs induces acute meningo-encephalitis with minor metastatic lesions. The disease is fatal in a relatively short time and is characterized by a rather typical succession of symptoms and a fairly characteristic temperature curve. The disease is produced by very small numbers of bacilli, and under standard conditions the period of survival is so uniform as to make possible quantitative or titrational experiments. Certain possible applications of the method are discussed.

FROM THE AUTHOR'S SUMMARY.

SUSCEPTIBILITY OF SWINE TO THE VIRUS OF HUMAN INFLUENZA. R. E. SHOPE and T. FRANCIS JR., J. Exper. Med. **64**:791, 1936.

Swine inoculated intranasally with the virus of human influenza alone present an ill defined mild and usually afebrile illness of short duration. Post mortem the anterior lobes of the lungs contain scant scattered areas of atelectasis. Transmission of the virus for five serial passages through two groups of swine failed noticeably to enhance its pathogenicity for this species. The disease produced in swine by infection with the virus of human influenza alone is clinically and

pathologically indistinguishable from that caused by infection with the virus of swine influenza alone. Transmission of the virus of human influenza from swine to swine by contact succeeded only in one of four attempts. Swine inoculated intranasally with a mixture of the virus of human influenza and Haemophilus influenzae-suis usually present a depressing febrile illness similar to mild influenza of swine. The pneumonia encountered in such animals at autopsy is similar to but less extensive than that seen in influenza of swine. In some animals H. influenzae-suis fails to become established, and the disease then seen is identical with that caused by the virus of human influenza alone. The virus of human influenza recovered after five serial transfers in swine was immunologically the same as that with which the experiments were begun.

FROM THE AUTHORS' SUMMARY.

ACQUIRED IMMUNITY IN RABBITS TO INTRANASAL INFECTION WITH TYPE I PNEUMOCOCCUS. T. E. WALSH and P. R. CANNON, J. Immunol. **31**:331, 1936.

A vaccine or an autolysate prepared from killed type I pneumococci protected rabbits against a dose of living pneumococci that was uniformly fatal in controls. Not less than five daily intranasal instillations of the vaccine were required. The infecting bacteria were administered by the same route. Instillation of nonspecific irritants (solution of formaldehyde, tannic acid, alum or paratyphoid bacilli) did not give protection. Ingestion of vaccine was not a factor in the immunization, because the intragastric route proved inferior to the intranasal. No antibodies were discovered in the circulating blood, and mice could not be protected with the blood serum or with the nasal tissue of the immunized animals, but the immunity was not merely local, as evidenced by the resistance of the intranasally vaccinated animals to intravenous injections of the living bacteria. The results indicate that the separation of local and general factors of immunity is exceedingly difficult when one is dealing with highly virulent micro-organisms in highly susceptible hosts.

I. DAVIDSOHN.

HAEMOPHILUS PERTUSSIS ON CHOCOLATE-BROWN AGAR. J. A. TOOMEY, W. S. TAKACS and K. H. RANTA, J. Infect. Dis. **59**:326, 1936.

A transfer of Haemophilus pertussis from blood to chocolate-brown agar medium caused a change in its antigenic activity. When cultures were subplanted on chocolate-brown agar shortly after their original isolation on blood agar and organisms thus obtained were used as an antigen to produce agglutinating serum, the titer of the latter was high not only against the homologous organisms grown on chocolate-brown agar but against the heterologous organisms grown on blood agar, possibly higher against the latter. The virulence of such organisms is diminished by a third at least.

When cultures that have been carried for a long time on blood agar (two years) are then subplanted on chocolate-brown agar and the organisms thus obtained used as an antigen to produce agglutinating serum, the titer of the latter is fairly high against the homologous organisms grown on chocolate-brown agar, but low against the heterologous blood-grown organisms. The virulence of such organisms is diminished by a third at least.

FROM THE AUTHORS' SUMMARY.

THE ELICITATION OF SPECIFIC PHAGE FROM AUTOCLAVED (LIFELESS) MATERIAL. A. I. KENDALL, J. Infect. Dis. **59**:340, 1936.

The experiments described in this article are believed to indicate that phage is not a living, autonomous ultramicrobe or a "living virus," that it is not an essential stage in a life cycle of bacteria and that it does not represent primarily a disturbance of bacterial metabolism. It seems to exhibit some properties in common with enzymes and also some properties not reconcilable with present concepts of enzymes.

FROM THE AUTHOR'S SUMMARY.

FAILURE OF STAPHYLOCOCCUS TO GROW FROM SMALL INOCULA. R. A. Q. O'MEARA and J. C. MACSWEEN, J. Path. & Bact. **43**: 373, 1936.

It is shown that the failure of Staphylococcus to grow from small inocula in routine laboratory mediums can be due to copper in the mediums. This may be introduced via bacteriologic peptones, in which it is often present in considerable quantities, or through the use of copper apparatus in preparing the medium. Copper salts in the concentrations in which they are liable to occur in mediums are inhibitory only after the medium has been heated and has stood for some time. The inhibition by copper conforms in this and other respects to the type of inhibition ordinarily met with in routine mediums. The value of the Huntoon-Wright method of preparing broth depends on the removal of the copper which is absorbed by the meat. Peptone also contains iron, which is simultaneously removed in this method of preparation. The variation in the copper and iron content constitutes a major factor in the variation of commercial peptones. It is desirable that manufacturers of peptone should give bacteriologists more detailed information concerning the composition of their products.

FROM THE AUTHORS' SUMMARY.

TITRATION OF VACCINIA VIRUS ON THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO. E. V. KEOGH, J. Path. & Bact. **43**:441, 1936.

Suspensions of the virus of vaccinia, inoculated in suitable dilutions, produce discrete lesions on the chorio-allantoic membrane of the developing embryo of the chick. The titer of a vaccinial suspension obtained by the enumeration of such lesions corresponds to that found by intradermal inoculation in the rabbit. Immune serums can be titrated in respect of their inactivating properties by the percental reduction effected in the number of such lesions. By this quantitative technic it is shown that the inactivating principle of immune serum combines with the particles of virus in vitro and that the union is partly dissociated by simple dilution. The percental inactivation of the virus of vaccinia effected by immune serum is independent of the concentration of the virus within the experimental range.

FROM THE AUTHOR'S SUMMARY.

STREPTOBACILLUS MONILIFORMIS. C. E. VAN ROOYEN, J. Path. & Bact. **43**:455, 1936.

Observations have been made on the epizootic infection of mice reported by Mackie, van Rooyen and Gilroy. A number of strains of the organism have been studied. These are identical and indistinguishable from the organism reported by Levaditi and by Strangeways and designated by the former as Streptobacillus moniliformis. The organism is similar to Bacillus piliformis (Tyzzer) and to certain "leptothrix" forms. The negative filtration experiments and the failure to find inclusion bodies have clearly differentiated the associated disease from ectromelia. Stained film preparations of Haverhillia multiformis supplied by Parker and Hudson have been examined and found to be morphologically indistinguishable from Streptobacillus moniliformis. One of the outstanding features of this bacterium is that it requires for growth a high proportion of blood serum in the culture medium. It may appropriately be placed in the tribe Haemophileae of the Bacteriaceae and recognized, as suggested by Parker and Hudson, as the representative of a new genus, the generic and specific names used by them being adopted: Haverhillia multiformis.

FROM THE AUTHOR'S SUMMARY.

DIRECT FERMENTATION OF DISACCHARIDES AND VARIATION IN SUGAR UTILIZATION BY STREPTOCOCCUS THERMOPHILUS. H. D. WRIGHT, J. Path. & Bact. **43**:487, 1936.

Certain strains of Streptococcus thermophilus utilize lactose and sucrose more readily than any other sugar. This is shown to be associated with a power to ferment these disaccharides, especially lactose, more readily than the constituent

monosaccharides. The results appear to be most easily explained by the assumption that the disaccharides are fermented without preliminary hydrolysis. The power of these strains to utilize sugars is shown to vary according to their recent environment in such a way as to suggest a quantitative fluctuation in enzymic constitution.

FROM THE AUTHOR'S SUMMARY.

Immunology

TETANUS TOXOID. F. G. JONES and W. A. JAMIESON, J. Bact. **32**:33, 1936.

Guinea-pigs that have received one or more injections of the alum-precipitated toxoid of tetanus and are infected within two months are protected against a massive dose of tetanus spores which kills normal guinea-pigs in approximately from three to four months. A massive dose of tetanus spores does not markedly accelerate the production of antitoxin in guinea-pigs that have been previously immunized with tetanus alum-precipitated toxoid.

FROM THE AUTHORS' SUMMARY.

QUANTITATIVE STUDIES ON ANTIBODY PURIFICATION. M. HEIDELBERGER and F. E. KENDALL, J. Exper. Med. **64**:161, 1936.

Quantitative data are given on the effect of changes in hydrogen ion concentration and of salt solutions of high concentration on certain immune precipitates obtained at a lower salt concentration. Advantage is taken of the shift in reaction equilibrium brought about by the salt in the case of pneumococcic carbohydrate-anticarbohydrate precipitates to make possible the preparation, in a single step, from unconcentrated serum, of antibody solutions in which up to 93 per cent of the total nitrogen is immune nitrogen. The method permits successive absorptions of a serum to be made with the same specific precipitate. A modification of Felton's dissociation procedure (Felton, I. D.: J. Immunol. **22**:453, 1932) is proposed, which yields highly purified antibody with precipitates which have been subjected to several successive salt dissociations.

FROM THE AUTHORS' SUMMARY.

COMPLEMENT FIXATION WITH PNEUMOCOCCIC CAPSULAR POLYSACCHARIDE. K. GOODNER and F. L. HORSFALL JR., J. Exper. Med. **64**:201, 1936.

Complement is not fixed by immune aggregates resulting from the interaction of pneumococcic capsular polysaccharide and type-specific immune horse serum, although under proper conditions the substitution of immune rabbit serum gives positive results. The negative results with immune horse serum are due to some poorly understood property of the specific antibodies rather than to some heterologous inhibitor present in the serum. It has been shown that with combinations of immune rabbit serum and polysaccharide, complement fixation is an adsorptive phenomenon conditioned on the surface exposure of the immune aggregates. A close parallelism to the selective adsorption of phosphatides by these immune aggregates is pointed out. In those instances in which complement is fixed, the phenomenon must be regarded as tertiary and conditioned by (*a*) union of antigen and antibody and (*b*) particulation. The general significance of complement fixation as applied to bacterial polysaccharides is discussed.

FROM THE AUTHORS' SUMMARY.

ACTIVE IMMUNIZATION OF GUINEA-PIGS WITH THE VIRUS OF EQUINE ENCEPHALOMYELITIS. H. R. COX and P. K. OLITSKY, J. Exper. Med. **64**:217 and 223, 1936.

Guinea-pigs given injections of amounts of the virus of active equine encephalomyelitis inadequate to induce protection against an intracerebral injection of 1,000 M. L. D. of the virus or more show no significant humoral antiviral bodies. The

latter are, however, regularly present in immune animals and are best demonstrated by adding their serum to low multiples of infective doses of the virus under optimal conditions of time and temperature (two and a half hours at 37 C.). Guinea-pigs immunized either with active or with inactive (formaldehydized) virus reveal no distinctive differences in the antiviral content of their serums.

In the active virus of equine encephalomyelitis and in the virus inactivated by solution of formaldehyde U. S. P. there is a sufficient amount of antigen without necessity of multiplication in the body to produce immunity in guinea-pigs against more than 1,000 and less than 10,000 M. L. D. (intracerebral). The antigenic capacity of both materials can be blocked to the same extent by the action of an appropriate amount of hyperimmune serum. The bearing of these findings on the mechanism of the immunity induced by virus inactivated with solution of formaldehyde is discussed. FROM THE AUTHORS' SUMMARIES.

NATURAL IMMUNITY TO PNEUMOCOCCUS TYPE III. M. F. SHAFFER and others, J. Exper. Med. **64**:281 and 307, 1936.

Cultures of two smooth strains, one virulent and the other avirulent, of Pneumococcus type III showed early loss of the capsule of the avirulent strain. The rough variants which dissociated from these strains possessed differences in morphology and growth, and the organisms obtained from reversion to the smooth type-specific forms of such rough variants resembled in virulence the original strains from which the rough variants were derived. Intravenous injection of the two strains showed that the degree of virulence varied with the state of the capsule; as capsular degeneration occurred the blood stream became clear.

FREDERICK STENN.

TISSUE CULTURE STUDIES ON HYPERSENSITIVITY TO TUBERCULIN. J. K. MOEN and H. F. SWIFT, J. Exper. Med. **64**:339, 1936.

A high degree of cellular sensitivity to tuberculin toxicity was demonstrated when explants from tuberculous animals were grown in medium containing that substance. Similar degrees of sensitivity were noted in cells derived from animals infected with either virulent or relatively lowly virulent strains of tubercle bacilli. The specificity of the sensitivity to tuberculin cytotoxicity was proved by testing with other cytotoxic bacterial materials. Tuberculin-sensitive cells grown in normal medium in vitro showed, when tested with tuberculin, persistence of this sensitivity through several transplantations during which many new generations of cells developed. There was depression of the initial growth energy of explants from animals during the toxic phase of the disease. During the healing stage the initial growth energy returned to normal although marked sensitivity to tuberculin persisted. The degree of cellular sensitivity to tuberculin in vitro did not parallel the acuity of the infectious process but represented a more or less permanent acquired characteristic impressed on the cell as a result of the infection.

FROM THE AUTHORS' SUMMARY.

TISSUE CULTURE STUDIES ON HYPERSENSITIVITY TO STREPTOCOCCUS. J. K. MOEN, J. Exper. Med. **64**:355, 1936.

Guinea-pigs infected with naturally pathogenic hemolytic streptococci (group C of Lancefield) acquired a low grade chronic disease characterized chiefly by purulent lymphadenitis. Cutaneous hyperreactivity to a crude extract of the streptococci invariably occurred during the course of this infection. The production of antibodies (precipitins and agglutinins) was studied. The extract of the streptococci when tested in vitro had a specific toxic effect on cells from infected animals; this was shown by microscopic evidences of injury and by quantitative inhibition of migration and growth. The specificity of the reaction was proved by testing with other cytotoxic substances. There was no parallelism between

the titers of skin hypersensitivity and humoral antibody. There was correlation between the degree of skin reactivity to the bacterial extract and the degree of sensitivity of splenic cells to the toxic action of the extract in vitro. Comparison of cellular sensitivity to tuberculin with cellular sensitivity to streptococcic extract in cultures of guinea-pig tissues showed that the former was more intense and was more persistent on prolonged growth in vitro.

FROM THE AUTHOR'S SUMMARY.

ACTIVE IMMUNIZATION TO SCARLET FEVER BY THE INTRACUTANEOUS METHOD. J. H. ROBINSON, J. Immunol. **31**:373, 1936.

An intracutaneous inoculation of the toxin of the streptococcus of scarlet fever was effective in changing the reaction in the Dick test from positive to negative in twenty-four subjects. The procedure had the advantage that it did not cause general reactions and brought about only a mild local reaction. About a twelfth of the number of skin test doses used in the subcutaneous method was injected.

I. DAVIDSOHN.

BLOOD GROUPING OF UPPER AUCANER BUSH NEGROES AND ALUKUYANA INDIANS IN DUTCH GUIANA. M. C. KAHN, J. Immunol. **31**:377, 1936.

Eighty-three per cent of 336 Bush Negroes of the Upper Aucaner tribe in Dutch Guiana, South America, were found to belong to group O and 17 per cent to group B. The number of persons tested represented about a third of the whole tribe. These Negroes are direct descendents of the slaves who were brought from the West African interior. Seventy pure blood Alukuyana Indians were determined to belong to group O.

I. DAVIDSOHN.

THE PRODUCTION OF HETEROPHILIC ANTIGEN BY CERTAIN BACTERIA AND PLANTS. G. E. ROCKWELL and H. C. VAN KIRK, J. Infect. Dis. **59**:171, 1936.

Bacillus mucosus is a high producer of heterophilic antigen, both endogenous and exogenous. Neisseria meningitidis and Haemophilus pertussis contain a moderate amount of heterophilic antigen. Freshly isolated strains of Haemophilis influenzae contain some heterophilic antigen.

Several strains of Brucella abortus, Neisseria gonorrhoeae and Bacillus acnei did not contain heterophilic antigen. Some evidence has been produced to show that such antigen may be capsular in origin as well as somatic. Spinach, beets and turnips contain some heterophilic antigen; asparagus, green beans, peas, rhubarb, celery, cabbage, lettuce, carrots, cauliflower and tomatoes do not contain any.

FROM THE AUTHORS' SUMMARY.

THE LOCAL POINTS OF DEFENSE AND THE PASSIVE TRANSFER OF ACQUIRED IMMUNITY TO NIPPOSTRONGYLUS MURIS IN RATS. M. P. SARLES and W. H. TALIAFERRO, J. Infect. Dis. **59**:207, 1936.

The mechanism of acquired immunity to Nippostrongylus muris (Nematoda) was studied by comparing the migration and development of the parasite in normal and hyperimmunized rats at short intervals during the previously unstudied first week of the infection and by passive transfer. (The rats were Bartonella-free.) In actively hyperimmunized rats the worms were retained and killed to a small extent in the skin and to a larger extent in the lungs; of those that migrated to the intestine, many were delayed, and on their arrival, the majority not only failed to grow or produce eggs but failed to remain. Passive transfer of immunity was obtained by giving intraperitoneal injections of serum from hyperimmunized rats in doses of 4.1, 4.5 and 6 cc. (per hundred grams of rat) to rats at the time of cutaneous infection with 3,500 or 4,000 larvae. The finding of stunted and immature worms in immune rats and the passive transfer of the

immunity, together with the finding of a precipitate around and in the gut of the worm in the skin and lungs of immune rats in preliminary histologic studies, indicate that the immunity to N. muris is essentially a local immunity in strategically placed organs, i. e., in the skin, lungs and intestine, and has to a large extent an antibody basis.

FROM THE AUTHORS' SUMMARY.

A MODIFIED TECHNIC FOR AGGLUTINATION TESTS IN BRUCELLA INFECTION. C. R. DONHAM and C. P. FITCH, J. Infect. Dis. **59**:287, 1936.

The modification consists in the use of a minimum amount of added water in the serum-antigen mixture. Thus there is a minimum amount of inhibition of agglutination from this source. Owing to the additional cost, the method with its relatively small advantage does not seem practical for routine diagnosis of brucelliasis in animals. Limited observation suggests that the modified technic may provide a better method of testing for undulant fever in man since in such work the slight additional cost per test does not need to be considered.

FROM THE AUTHORS' SUMMARY.

THE NATURE OF THE BACTERIAL SPECIFIC INTRADERMAL ANTISERUM REACTION. L. FOSHAY, J. Infect. Dis. **59**:330, 1936.

In tularemia the bacterial specific intradermal antiserum reaction is due to an antigen-antibody reaction involving only the species-specific polysaccharide. Indirect evidence indicates that the same or a similar mechanism is responsible for the identical type of bacterial specific reaction in certain other infectious diseases.

Since the production of positive reactions in patients is dependent on the existence in their skins of that part of the antigenic complex which determines the species-specificity of the invading parasite, the reaction serves as a confirmatory diagnostic test of great precision and accuracy. Differentiation of the type or strain of the infecting species is possible in certain diseases. In some diseases which confer prolonged immunity after recovery, diagnoses can be made in retrospect many years after complete convalescence. In such diseases the tests alone cannot be expected to distinguish between past and existing infections. On the other hand, it is possible in this group of diseases to select, by this method, the immune persons from a given sample of the population. In respect to infections which do not confer lasting protection, the test may be useful as a measure of the duration of immunity. Early experience suggests that it may serve the same purpose in relation to artificially acquired immunity. Because of the high degree of specificity, the test appears to be a valuable method for testing relations between alleged microbic causes of disease and these diseases in their classic clinical forms. Even with the present limited experience, the test seems to be the most accurate means available to determine which polyvalent antistreptococcus serum is most suitable for a given patient.

Serums for test purposes should be strictly species-specific. They are best made by using only recently isolated virulent strains. In general, the available commercially produced serums are unreliable for test purposes. In performing these tests it is imperative that strictly normal serums should be injected simultaneously as controls.

FROM THE AUTHOR'S SUMMARY.

PARABIOTIC TWINS AND CELLULAR INDIVIDUALITY. M. R. IRWIN and R. T. COLE, Proc. Soc. Exper. Biol. & Med. **33**:566, 1936.

Hybrids between male pearlnecks and female ring doves were back-crossed to doves of the latter (domestic) variety. Parabiotic unions were made between two young back-crossed doves. Each member of the pair of parabiotic twins showed antibodies against the red cells of the other.

A. S. WIENER.

TOXINS OF THE STAPHYLOCOCCUS, WITH SPECIAL REFERENCE TO THE ESTIMATION OF POTENCY. F. G. MORGAN and J. J. GRAYDON, J. Path. & Bact. **43**:385, 1936.

Many toxic filtrates prepared from single strains of staphylococci on artificial mediums under prescribed conditions show well defined differences in their combining affinity for antitoxin. By means of two type serums, S. 35 and R. 27, toxic filtrates have been classified into two groups. Frequent reference was made to international standard antitoxin. Two components of a type with distinct combining properties have been demonstrated. It is not clear whether both are derived originally from a single entity formed during cultivation or whether two separate and distinct entities are present from the beginning. In the former case relative avidity of toxin and antitoxin may explain the phenomena. However explained, the two components act separately and for practical purposes constitute two parts of the toxic filtrate. We have named them a_1 and a_2 toxins, and both are present in the majority of toxic filtrates made from individual strains from human sources. All the toxic filtrates so prepared possess a_1 toxin; the majority also have a smaller but definite amount of a_2 toxin. Both toxins appear to be antigenic; both are hemolytic for rabbit red cells, and both cause necrosis of the skin of guinea-pigs. Owing to the small proportion of a_2 toxin in toxic filtrates, demonstration of the lethal effect of this toxin in rabbits was not practicable. The *a* types of toxin are differentiated from the *B* type of Glenny and Stevens. A toxic filtrate containing both rabbit cell and sheep cell lysins is described. The antitoxins specific for each *a* toxin are partially separable by methods of fractionation. When toxic filtrates containing both components are titrated against a single antitoxin, the hemolytic test reveals that neutralization of the separate components is frequently indicated by the appearance of two end-points, one showing partial hemolysis (a_1) and the other complete inhibition of hemolysis (a_2). This effect is paralleled in titrations on the skin. Owing to the dual nature of some *a* toxins, ordinary methods of standardization give rise to gross errors in estimating unitage.

FROM THE AUTHORS' SUMMARY.

THE BLOOD GROUP A IN MAN AND IN THE RABBIT. O. THOMSEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **87**:335, 1936.

The human blood group factor A is a complex of fractions. Rabbits can be roughly divided into two groups: one with some of the A fractions and another without, but there are transitions between the two groups. No rabbit possesses all these fractions (the factor is present in its entirety in man and probably in anthropoid apes but in no other animals), and therefore all rabbits can produce specific antibodies for those fractions which they do not possess in their tissues. The less of the A antigen there is in the rabbit the better is the group-specific antibody response. On the other hand, the rabbits well endowed with fractions of A respond better than the others in the development of species-specific human antibodies (for blood group O). The human blood group factors A and AB contain in common with sheep red cells a fraction of the Forssman antigen and in addition another form of the Forssman antigen which is present in all the blood groups (also in O). The difference between the A_1 and A_2 subgroups lies not in the A property but in the Forssman antigen (the one present only in group A and in sheep red cells), which antigen may be either qualitatively or quantitatively different in the A_2 subgroup.

I. DAVIDSOHN.

BLOOD-CEREBROSPINAL FLUID BARRIER AND ANAPHYLACTIC ANTIBODIES. A. PONOMAREW and A. KANAREVSKAJA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **87**:518, 1936.

Dogs and rabbits were sensitized with horse and with chicken serums. Guinea-pigs were passively sensitized with the serums of the actively sensitized dogs and rabbits. Twenty-four hours later an intravenous injection of the homologous antigen produced in the guinea-pigs a typical anaphylactic shock. Guinea-pigs given injections of the cerebrospinal fluids of the actively sensitized animals and subsequently injections of the homologous antigen showed no manifestation of

anaphylaxis. The blood-fluid barrier is as efficient in preventing the passage of anaphylactic antibodies as in preventing the passage of hemolysins, agglutinins, etc.

I. DAVIDSOHN.

GROUP-SPECIFIC DIFFERENTIATION OF THE ORGANS OF THE RABBIT. L. A. SCHWARZMANN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **87**:525, 1936.

Schwarzmann undertook to reinvestigate the distribution of the A and B factors in the organs of the rabbit. According to previous investigators, the A factor is present in different organs and in the serum of many rabbits; the factor B, only in the erythrocytes, not in the organs. Schwarzmann employed the simple method of adsorption of iso-agglutinins from human serum with suspensions of the different organs. With fifty rabbits he found the B factor in about 57 per cent, the A factor in 2.3 per cent, the AB factor in 16 per cent and neither A nor B in 16 per cent. The differentiation of the groups is not so clearcut as in man; in some organs the group factors found were different from those in other organs of the same animal. The erythrocytes behaved like the other tissues, which is contrary to the findings of some of the previous investigators.

I. DAVIDSOHN.

SEROLOGIC SPECIFICITY OF PATHOLOGIC TISSUES. L. HIRSZFELD and W. HALBERÓWNA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:41, 1936.

By immunization of rabbits with raw and boiled aqueous extracts and with alcoholic extracts of human and of equine pus and of human and bovine tuberculous caseous tissue, immune serums were produced. By means of complement fixation tests with the immune serums and the corresponding extracts, antigenic relations were found between the tuberculous caseous tissues of man and beef and the pus of man, but none between the tissues named and the pus of the horse. The antigens of these tissues were highly thermoresistant (coctostabile) and alcohol soluble. By proper technical procedures it was possible to separate platelets from leukocytes and to produce corresponding specific immune serums. The antigen of the platelets was alcohol insoluble and thermolabile (coctolabile), and the antigen of the leukocytes was alcohol soluble and thermostabile (coctostabile).

I. DAVIDSOHN.

PHOTOMETRIC STUDY OF SPECIFIC PRECIPITIN. T. J. KUROTCHKIN and C. E. LIM, Chinese M. J., supp., 1936, p. 261.

The use of Pulfrich photometer (Zeiss) in the study of the specific precipitin reaction shows that the general course of the reaction may be tentatively divided into four separate zones, each characterized by a definite behavior. The Pulfrich photometer permits a most accurate determination of optimal proportions of antibody-antigen mixtures associated with the formation of visible precipitate. The reaction occurring at the zone of antigen deficiency is found to follow the law of the direct proportion between the antigen concentrations and the turbidity of the mixtures.

FROM THE AUTHORS' SUMMARY.

RECOVERY OF ANTIBODIES. HARUMI SUMA, Arb. a. d. Med. Fak. Okayama **4**:465, 1934.

The recovery of antibodies from sensitized antigen is most effectively carried out at certain temperatures, depending on the medium of solution.

Medium of Solution	Temperature, C.
Distilled water	55
Hypotonic saline solution (0.034 per cent physiologic solution of sodium chloride)	60
Physiologic solution of sodium chloride	65

In the dissociation of antibody from agglutinated heated as compared with agglutinated native colon bacilli it was found that with increase of the temperature up

to a limit of 100 C. (three hours) the affinity of immune substance to antigen is weakened, and therefore a larger amount is recovered from bacilli heated to 100 C. (one hour) than from native bacilli or those heated only to 60 C. (one hour). The results also indicate that the reversibility of the antigen-antibody reaction is favored by acid salts and salts containing bivalent ions.

FREDERICK STENN.

THE COURSE OF PASSIVE IMMUNITY AFTER TREATMENT WITH ANTI-DIPHTHERIA HORSE SERUM. E. MADSEN, Acta path. et microbiol. Scandinav. **13**:103, 1936.

Blood from fifteen patients with diphtheria was studied for (*a*) diphtheria antitoxin by the Jensen method (Jensen: *Acta path. et microbiol. Scandinav.*, supp. 14, 1933) and for (*b*) antihorse precipitins. Five of the patients were given the antitoxin intravenously, seven intramuscularly and three in both ways. While in rabbits there was a characteristic sudden very rapid drop of the antitoxin, beginning around the sixth or seventh day after the injection of the antitoxin and about two days before the appearance of the antihorse precipitins, in man the disappearance of the antitoxin was gradual. In four patients serum disease was observed on the eighth (two cases), eleventh and fourteenth days after the injection of the antitoxin. In only one of them was an accelerated loss of the antitoxin noted, corresponding with the onset of serum disease. In only two of them did precipitins appear in the circulation. In none of the patients without serum disease did precipitins appear. Considerable amounts of antitoxin were found in the circulation as late as four weeks after its injection, indicating longer duration of the antitoxic immunity than is commonly reported. The intravenous route is recommended because, after intramuscular injection, it takes a few days before the maximum quantity of antitoxin is present in the circulation.

I. DAVIDSOHN.

THE BLOCKADE OF THE RETICULO-ENDOTHELIAL SYSTEM IN IMMUNIZATION AS REVEALED BY CONGO RED TESTS. J. PHILIPSON, Acta path. et microbiol. Scandinav. **13**:314, 1936.

The congo red test of the blood in rabbits indicates that in the course of immunization with paratyphoid antigen the antigen blocks the reticulo-endothelial system in the same way that india ink does. In normal rabbits no such blockade seems to take place. It appears that the elimination of congo red from the blood depends to a large extent on the state of the reticulo-endothelial system. As a congo red index Philipson has used the percentage of congo red absorbed sixty minutes after an intravenous injection of 10 mg. per kilogram of body weight.

I. DAVIDSOHN.

SPECIFIC ANTIBODIES AGAINST THE AGENT OF CHICKEN LEUKOSIS. E. UHL and others, Acta path. et microbiol. Scandinav. **13**:394, 1936.

While the serum of normal ducks does not neutralize the leukotic agent in chicken blood, the serum of ducks immunized with leukotic blood contains "antibodies" against this agent. Ducks immunized with normal chicken blood have no such "antibodies."

I. DAVIDSOHN.

Tumors

EARLY STAGES OF THE TRANSPLANTATION OF THE EHRLICH-PUTNOKY TUMOR INTO MICE AND RATS. F. R. SELBIE, Am. J. Cancer **28**:530, 1936.

The Ehrlich-Putnoky tumor is a sarcoma or a very anaplastic Ehrlich carcinoma. Its rapidity of growth probably explains the ease with which it lends itself to heteroplastic transplantation. Its growth in the rat is a temporary proliferation, unaccompanied by sarcomatization of the normal connective tissue.

Heterologous transplantation is of little importance in the study of cancer. General conclusions based on results of experiments in which heterologous tumors have been used are valueless. FROM THE AUTHOR'S SUMMARY.

POSSIBLE EFFECT OF OIL OF GAULTHERIA IN THE DIET OF MICE SUSCEPTIBLE TO SPONTANEOUS CARCINOMA OF THE MAMMARY GLAND. L. C. STRONG, Am. J. Cancer **28**:550, 1936.

The daily administration of true oil of gualtheria to mice bearing spontaneous tumors of their mammary glands has a pronounced effect on the clinical course and histologic appearances of the tumors. In early stages, the connective tissue of the tumors seems to be materially enhanced by such treatment. It is suggested that similar studies may lead to fundamental conclusions regarding the mechanics of the origin of spontaneous tumors and the control of the disease, at least in experimental animals. FROM THE AUTHOR'S SUMMARY.

PROBABLE HODGKIN'S DISEASE IN A DOG. L. K. STALKER, C. F. SCHLOTTHAUER and W. H. FELDMAN, Am. J. Cancer **28**:595, 1936.

Authentic cases of Hodgkin's disease in animals are extremely rare, but the gross and the microscopic picture observed in this dog exhibited all of the essential criteria of Hodgkin's disease in man. FROM THE AUTHORS' SUMMARY.

NUCLEAR SIZES IN GROWTH DISTURBANCES AND NEOPLASMS. W. E. EHRICH, Am. J. M. Sc. **192**:772, 1936.

This report is based on the measures of 220,000 nuclei of 230 specimens, half of which showed disturbances of growth. In all the tissues studied, normal as well as pathologic, several classes of nuclei were found in which the sizes were in the proportions of Heidenhain's law of growth. The sizes of the nuclei of normal tissues are by no means constant; they vary not only in the same organ in different persons but in different organs of the same person. In physiologic variations, apparently functional influences play the main rôle. In hypertrophy and atrophy, several mechanisms are involved. In certain organs the size of the nuclei increases or decreases in the proportions of Heidenhain's law with a shift in the proportion of the smaller to the larger cells or vice versa (liver, prostate). In other organs the number of cells may increase or decrease with or without change of their size (lymphatic and lymphoid tissue). In still others it concerns mainly a simple swelling or shrinkage outside of the proportions of Heidenhain's law (breast, thyroid). Which of these avenues is chosen depends largely on the regenerative ability of the tissues and probably on the ability of the cells to swell or shrink functionally. Benign tumors are characterized by cells larger than their mother cells. The enlargement is due to simple swelling and not to true growth. Malignant tumors are characterized by cells which are double or fourfold the size of their mother cells and which probably arise by deviating from normal growth during cell division. The malignant tumor cell is not an embryologic cell but a special cell that occurs in malignant tumors only. The double or quadruple size of the cell is an anatomic expression of the anaplasia or kataplasia of malignant tumors so long searched for.

FROM THE AUTHOR'S SUMMARY.

THE MECHANISM OF A HITHERTO UNEXPLAINED FORM OF NATIVE IMMUNITY TO THE TYPE III PNEUMOCOCCUS. A. R. RICH and C. M. MCKEE, Bull. Johns Hopkins Hosp. **59**:171, 1936.

Rabbits given intracutaneous injections of seven encapsulated mouse-virulent strains of type III pneumococci showed marked resistance to the infection when their temperature was elevated to from 104 to 106 F. by keeping them in an incubator before and after the infection. In vitro these strains were markedly

sensitive to such temperatures. With the temperature elevated to fever levels capsular alterations appeared early, and the organisms were avidly phagocytized.

FREDERICK STENN.

PRIMARY CARCINOMA OF THE SEMINAL VESICLES. A. MCNALLY and F. M. COCHEMS, J. Urol. **36**:532, 1936.

Sixteen instances of primary carcinoma of the seminal vesicles have been reported in the literature, of which only four are definitely proved. In the case herein reported the patient's chief complaint was hematuria. The diagnosis of primary carcinoma of the right seminal vesicle was made on the basis of hematuria and the presence of a hard mass in the right seminal vesicle. The tissues were radically excised, and the clinical diagnosis was confirmed by histologic examination. Later the prostate was removed and found free from carcinoma. An additional interesting histologic observation was that of tuberculosis, apparently an activated latent infection associated with old tuberculosis of the spine. The patient is free from clinical evidence of recurrence or metastasis more than two years after operation. This seems to be the only case of primary carcinoma of the seminal vesicle on record in which the patient is alive and free from symptoms of the tumor two years after radical excision.

FROM THE AUTHORS' SUMMARY.

COMPARATIVE EFFECTS OF ROENTGEN RAYS AND NEUTRONS ON NORMAL AND TUMOR TISSUE. J. H. LAWRENCE, P. C. AEBERSOLD and E. O. LAWRENCE, Proc. Nat. Acad. Sc. **22**:543, 1936.

Per unit of ionization, neutrons are much more effective than roentgen rays in destroying normal mice in vivo and sarcoma 180 in vitro. The preliminary results indicate that neutrons are three times as effective in destroying normal mouse tissue and four times as effective in destroying sarcoma 180 in vitro.

FROM THE AUTHORS' SUMMARY.

THE AMINO-ACID LYSINE AS A FACTOR IN MALIGNANT GROWTH. C. VOEGTLIN and others, Pub. Health. Rep. **51**:1429 and 1536, 1936.

A diet composed essentially of 70 per cent ground wheat and 30 per cent whole milk powder promotes normal growth in young rats and rapid growth of spontaneous mammary carcinomas in mice. If the milk powder of this diet has been subjected to heat under the specified conditions, the resulting diet is inadequate for normal growth, and malignant growth as a rule is greatly inhibited. This inhibition of normal and of malignant growth is removed by administering lysine. An adequate supply of lysine in utilizable form is therefore necessary for rapid growth of the malignant tumor used in these experiments.

Normal growth of young mice and growth of spontaneous mammary carcinomas of adult mice are inhibited by a diet containing gliadin as the source of protein. The addition of lysine renders this diet adequate for both normal and malignant growth. Similar experiments with a diet in which glutenin takes the place of gliadin indicate that normal and malignant growth are not inhibited. Since gliadin is known to be deficient in the indispensable normal growth factor, lysine, whereas glutenin is a complete protein, the conclusion is reached that lysine is a factor essential to the growth of the mammary carcinoma.

FROM THE AUTHORS' SUMMARIES.

EFFECT OF GLUTATHIONE ON MALIGNANT GROWTH. C. VOEGTLIN, J. M. JOHNSON and J. W. THOMPSON, Pub. Health Rep. **51**:1689, 1936.

The rate of growth of a spontaneous mammary carcinoma can be controlled by means of a diet deficient in cystine and methionine.

Following a period of inhibition of the growth of the tumor, the administration of either cystine or glutathione exerts a striking stimulative action on its growth.

FROM THE AUTHORS' CONCLUSIONS.

THE RESPONSE OF THE MAMMARY GLAND TO OVARIAN HORMONES. IAN MACDONALD, Surg., Gynec. & Obst. **63**:138, 1936.

For a time continuous administration of estrogen to rabbits which had been castrated produced generalized overgrowth and ramification of the ductal system in the mammary gland with almost no acinar growth. When the administration of the hormone was continued for one hundred days atypical histologic variations appeared in the ductal epithelium, often resembling borderline neoplastic changes, with little gross alteration of the glands. When stimulation by estrogen was continued for six months or more, there was almost complete regression of the duct cell hyperplasia, and the mammary glands returned to a nearly resting state, indicating that in rabbits stimulation by estrogen is confined to certain limits, past which a refractory state is set up. Widening of the ducts is not a prominent feature. Estrogen and extract of corpus luteum in combination produced both ductular and acinar overgrowth, early secretion and distention of the ducts. MacDonald believes that the dilatation of the ducts was probably the result of mechanical distention by the secretion and not a specific effect of the estrogen and that, on the basis of present evidence, estrogen and progestin do not produce specific pathologic lesions, cystic disease and adenosis.

FROM THE AUTHOR'S CONCLUSIONS.

ASSOCIATION OF CHROMAFFIN TUMORS WITH NEUROFIBROMATOSIS. D. B. ROSENTHAL and R. A. WILLIS, J. Path. & Bact. **42**:599, 1936.

Bilateral chromaffin tumor of the adrenal gland associated with cutaneous neurofibromatosis was observed in a man who died from pulmonary tuberculosis. The association of neurofibromatosis with bilateral paraganglioma of the adrenal glands is too frequent to be fortuitous; an essential relationship must exist between the two conditions. Special attention is drawn to the presence of nerve cells and nerve fibers in the tumors in this case and to the chromaffin properties of some of these nerve cells.

FROM THE AUTHORS' SUMMARY.

THE CONSTITUENT ELEMENTS OF A METASTATIC ADENOCARCINOMA OF THE DURA MATER: RÔLE OF THE MICROGLIA. G. MARINESCO and M. GOLDSTEIN, Ann. d'anat. path. **12**:101, 1935.

Marinesco and Goldstein examined a metastasis from a carcinoma of the breast to the dura mater, which had compressed but not invaded the brain. Microscopic examination of sections prepared with various stains revealed microglia, which apparently had reached the tumor via the blood vessels. The presence of microglia in a metastatic tumor which itself contains neither nerve tissue nor glia is further support for the theory that the microglia is of mesodermal origin.

PERRY J. MELNICK.

EOSINOPHILIA IN MALIGNANT TUMORS. J. PAVIO, M. LEVRAT and A. GUICHARD, Ann. d'anat. path. **12**:113, 1935.

In a 56 year old man with a perirenal reticulosarcoma, there was found persistent eosinophilia up to 76 per cent. Eosinophils were also present in abundance in the connective tissue stroma of the tumor. Only ten cases of eosinophilia associated with a malignant tumor are recorded. Among various hypotheses, the authors consider the most reasonable one to be that there is a sensitization to the neoplastic protein.

PERRY J. MELNICK.

RETICULO-ENDOTHELIOMA OF THE GLUTEAL REGION. C. LENORMANT and P. MOULONGUET, Ann. d'anat. path. **12**:425, 1935.

The authors report a "reticulo-endothelioma" of the right gluteal region in a 37 year old woman. It was excised surgically. [From the description and from two good photomicrographs the tumor appears to be an angio-endothelioma derived from vascular endothelium and not from reticulo-endothelium.]

PERRY J. MELNICK.

FIBROMYXOMA OF THE TENDON OF THE PSOAS MINOR. P. L. MIRIZZI, Ann. d'anat. path. **12**:807, 1935.

Mirizzi presents a contribution on retroperitoneal tumors in the form of a report of a fibromyxoma which developed in the tendon of the right psoas minor muscle. The tumor was removed surgically. It occurred in a 24 year old woman with symptoms of pain in the right lower quadrant of the abdomen, radiating downward to the extremity. Physical and roentgen examinations revealed a retroperitoneal tumor.

PERRY J. MELNICK.

PULMONARY CHONDROMA. L. JAEGER, Ann. d'anat. path. **12**:811, 1935.

A discussion of the pathologic anatomy of chondroma of the lung is followed by a description of five cases. The presence of organoid structures, cysts, glandular elements, etc., in these cases as well as in others reported leads the author to assign the tumor to the group of congenital malformations in the sense of the hamartoma of Albrecht.

PERRY J. MELNICK.

MEDICOLEGAL ASPECTS OF CANCER. A DIETRICH, Ztschr. f. Krebsforsch. **42**:251, 1935.

Four factors must be considered in the genesis of any cancer: the general constitutional predisposition; the focus of origin; the release of the growth impulse at this focus, and the mutation by which the cancer cells achieve and maintain their property of continuous and unrestricted growth. In forensic medicine, the factor of constitutional predisposition enters only into the field of occupational cancer, where it may be the sole element of importance, as in the cancers caused by aniline or arsenic, or it may be associated with the also occupational development of a local focus, as in cancers caused by tar and roentgen rays. Trauma is concerned with the appearance of a local focus or with the release of the growth impulse at a focus in which the cancerous tendency is already present but latent. Its importance in any given case depends on the character of the injury and of the resultant tissue reaction, and consideration must be given to the intervals of time involved. To be considered as of significance, the injury must have caused appreciable and lasting change in the tissue affected; frequently the immediacy of the appearance of cancer may exclude the injury as a causative factor, as in a case of gastric cancer following an injury after the interval of only a few months. Here a chronic process with prolonged regenerative changes, needing a much longer time, must be regarded as essential. To a certain extent, provided persistent alteration in tissue occurs, the probability of development of cancer increases with the interval of time. A causal association can seldom be legitimately maintained in a case of comparatively trivial injuries, of transient character. However, even with these there may occasionally be such an association, as in old healed burns, in which the predominant factor appears to be the later development of a constitutional predisposition. When malignant growth follows shortly after an injury, the effect is presumably that of a release of growth impulses already present but latent. As yet there is no evidence that injury can affect the progress of a malignant growth already present, except in those rare cases in which mechanical violence might cause the discharge into the circulation of large numbers of tumor fragments.

H. E. EGGERS.

A DIAGNOSTIC REACTION FOR CANCER. J. ČÍŽEK, Ztschr. f. Krebsforsch. **42**:311, 1935.

There is described here a modification of the Fuchs diagnostic reaction for cancer, which appears to simplify that test. The Fuchs reaction depends on the presence, in cancerous serum, of abnormal proteolytic ferments. To demonstrate the presence of these, Čížek uses the ability of Bacillus coli to break up inter-

mediate cleavage products into the amino-acids. He tests for these by the indol reaction for tryptophan. Taking fibrin which has been carefully tested for the absence of tryptophan in experimental conditions, he incubates this along with the serum to be tested, and later, for a longer period, after inoculation with carefully washed colon bacilli. With this technic he obtained correctly positive results in 90 per cent, and correctly negative results in 94 per cent, of his tests.

H. E. Eggers.

Morphologic Alterations of Cervical Cancers in the Course of Irradiation. W. W. Ossinskaja, Ztschr. f. Krebsforsch. **43**:66, 1935.

The usual explanation of the greater resistance of squamous cell carcinoma of the cervix to subsequent irradiations is that as a result of the first exposure there has occurred a greater differentiation of the tumor. Ossinskaja finds in his material that the reverse is true, that as far as structural relations are concerned these are less developed. However, as regards individual cells, this was not entirely the case, as many showed closer approximation to maturity than did those of the original cancer. He agrees that while greater differentiation may occur after irradiation, this is not constant, and he believes that the acquired insensitivity is due to changes in the cells which are not necessarily reflected in morphologic alterations.

H. E. Eggers.

Trichinosis and Cancer. W. Schmidt-Lange, Ztschr. f. Krebsforsch. **43**:264, 1936.

Although in a number of cases carcinoma in man has been reported in sequence to trichinotic infection, the etiologic relationship is still far from being definitely established. Schmidt-Lange reports the development of a metastasizing sarcoma of the liver in one of several mice infected with the parasite. From the facts that primary sarcoma of the liver in a mouse is very rare, that the mouse was not of a strain hereditarily predisposed to malignant change and that the time relationships were such as to support the idea of causal relationship, he believes that this occurred in this instance. As to possible mechanisms, he mentions two: (1) irritation of the liver by passage through it of large numbers of young parasites and (2) the action of metabolic products of parasitic growth.

H. E. Eggers.

Fate of Cancer Cells Lodged in the Lung. G. F. W. Kost, Ztschr. f. Krebsforsch. **43**:291, 1936.

Kost found demonstrable intravascular tumor cells in five of twelve cases of carcinoma of the lung. In part these were embedded in fibrin and showed evidence of disintegration. Some were well preserved. To explain lodgment without the establishment of metastases, there are several possibilities: very recent lodgment; restriction or destruction, as by fibrinous encapsulation; adverse physiochemical relationships; cytolytic action of the serum, and a restrictive effect of the organ in which lodgment has occurred.

H. E. Eggers.

Technical

Vacuum-Dried Complement in the Routine Wassermann Reaction. F. Boerner and M. Lukens, Am. J. M. Sc. **192**:272, 1936.

Complement dehydrated from the frozen state and sealed in vacuo (lyophil complement), when stored in the refrigerator at from 8 to 10 C., retained its full hemolytic activity and fixability for a period of twelve months. The titrations of both the complement and the hemolysin gave approximately constant results over this entire period. Thereafter deterioration was detected. This complement was used in the Wassermann test of 12,175 specimens of blood and 675 specimens of

spinal fluid and proved in all respects equivalent to fresh complement. The authors have discontinued the maintenance of a guinea-pig colony because of the greater convenience and uniformity of this type of complement.

FROM THE AUTHORS' SUMMARY.

A METHOD FOR RESTORING THE BODY AFTER AUTOPSY. E. D. PEASLEY, J. A. M. A. **107**:1378, 1936.

In restoring the great vessels for the undertaker after autopsy Peasley employed the device of dividing the innominate, the left common carotid, the left subclavian and the external iliac arteries, tying into each a glass cannula, and by means of glass connecting U tubes and rubber tubing established access to these vessels with rubber tubing extending through the abdominal incision. If the carotid arteries were to be examined in the neck, the tubing was made correspondingly longer. The cost of the material was about 50 cents in each case. The use of this method in 100 bodies gave gratifying results and won the cooperation of the undertaker.

FREDERICK STENN.

A HIGH SPEED VACUUM CENTRIFUGE SUITABLE FOR THE STUDY OF FILTERABLE VIRUSES. J. H. BAUER and E. G. PICKELS, J. Exper. Med. **64**:503, 1936.

A high speed centrifuge is described in which the speed is limited only by the strength of the material of which the rotor is made. It carries sixteen tubes, each of which conveniently accommodates 7 cc. of fluid. The centrifuge operates in a very high vacuum and therefore requires only a small amount of driving energy. The arrangement has been found to eliminate the possibility of producing injurious frictional heat. The rotating parts are supported by an air-bearing and are driven by compressed air. The centrifuge has been successfully operated at a speed of 30,000 revolutions per minute, representing a maximal centrifugal force in the fluid of 95,000 times gravity. Celluloid tubes used for centrifugation of fluid at high speeds are described.

FROM THE AUTHORS' SUMMARY.

SOURCES OF ERROR IN THE LABORATORY DIAGNOSIS OF AMEBIASIS. W. DE YOUNG, J. Lab. & Clin. Med. **21**:1149, 1936.

Since the characteristics of trophozoites of Endamoeba histolytica and Endamoeba coli in primary cultures are so often very similar and therefore indistinguishable, the use of primary cultures in the diagnosis of amebiasis is a source of error and, therefore, such cultures should be used with caution as a means to a final diagnosis. Fixation and staining of trophozoites from cultures is not an accurate means of diagnosis, since the nuclear changes which occur in cultures result in rather marked similarity between the nuclei of E. histolytica and E. coli. The diagnosis of amebiasis by means of direct smears is certain only when (1) cysts, (2) trophozoites containing ingested red cells or (3) typical trophozites of E. histolytica are present. When the samples contain inactive or sluggish trophozoites, fixation and staining should be used or other samples obtained. For the present, at least, examination of samples should be confined to material furnished by a natural bowel movement. It is quite possible that the use of saline purges may increase the number of cases of amebiasis in which the diagnosis is made on a single examination, but its use is not recommended since, in the experience of De Young, it may cause confusion. Following a saline purge, E. coli becomes quite active and tends to assume the characteristics found in the pathogenic form.

FROM THE AUTHOR'S SUMMARY.

STATISTICAL INTERPRETATION OF DATA IN EXPERIMENTAL TUBERCULOSIS. W. N. BERG, Tubercle **17**:496, 1936.

A simple statistical method for the interpretation of data on experimental tuberculosis is described, the purpose being to show how to test the significance of

the difference between two means (or averages) by a method that requires little knowledge of mathematics beyond arithmetic and to apply this test to some recent "immunization" experiments described in the literature. It can be used to test the significance of the difference between mean weights of spleens, mean numbers of tubercles and mean numbers of days, weeks or months of life after infection. A table illustrating the method is included.

H. J. Corper.

A High Titer of Agglutinins in Serum of a Dangerous Universal Donor. M. Muller and E. Balgairies, Compt. rend. Soc. de biol. **121**:1447, 1936.

A 24 year old patient with pernicious anemia was given a transfusion of group O blood. After twenty minutes a severe reaction set in, which lasted about an hour. The titer of the agglutinin of the donor's serum against A cells was 1:2,048, and that against B cells was 1:1,024. The patient belonged to group A.

A. S. Wiener.

The New Kolmer Modification of the Wassermann Reaction. K. H. Bau, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:348, 1936.

The older Kolmer technic (1922) proved somewhat inferior to the official German modification of the Wassermann test in 1,300 specimens. The superiority of the latter was evidenced by the detection of 20 cases established to be instances of syphilis in a total of 33 cases in which the test was positive while the Kolmer test was negative. In the same group 6 cases were clinically suspected of being cases of syphilis, and in 5 there was no clinical evidence of the disease. On the other hand, of 21 cases in which the Kolmer reaction was positive and the German technic negative, only 8 were established clinically to be instances of syphilis; in 6 the nature of the disease was doubtful and in 5 syphilis was clinically absent. In contrast to the older technic, Kolmer's new modification with its new antigen and larger amount of serum proved superior to the German official technic in 282 tests. The new Kolmer test was positive and the German modification was negative in 10 cases, of which 6 were clinically established to be instances of syphilis, 2 were suspected of being cases of syphilis, 2 were lacking available clinical data and none could be definitely excluded as instances of syphilis. Of 8 cases in which the new Kolmer test was negative and the German official modification positive, 3 were clinically established to be instances of syphilis, 4 were suspected and 1 showed no evidence of syphilis. Bau recommends the new Kolmer modification on account of its higher sensitivity, specificity and quantitative character.

I. Davidsohn.

The Influence of the Antigenic Extract on the Sensitiveness of the Wassermann Reaction. R. Schmiemann, Ztschr. f. Immunitätsforsch. u. exper. Therap. **89**:90, 1936.

The usual extract of beef heart was prepared with 5 volumes of alcohol and 1 part of tissue. The accepted procedure is to dilute the aforementioned extract three times with alcohol. Schmiemann observed that diluting it thirty times increased its sensitiveness in the complement fixation test for syphilis. This increase was particularly noticeable when the two dilutions were rapidly prepared. The differences between the rapid and the fractional dilution of the extracts were absent in the 1:30 dilution. The advantage of the higher dilution was especially apparent with weakly positive serums. Complement was fixed in the presence of smaller amounts of serum. The optimal concentration of cholesterol in the higher dilution of the antigen depended on the rapidity with which the extract was diluted. In fractionally diluted extracts the sensitiveness decreased, and in rapidly diluted extracts it increased with increasing concentrations of cholesterol. In a small number of syphilitic cerebrospinal fluids the higher dilution of the antigen proved more sensitive than the usual 1:3 dilution.

I. Davidsohn.

A SIMPLE MODIFICATION OF RUSSELL'S DOUBLE SUGAR MEDIUM. S. H. ZIA, Chinese M. J., supp., 1936, p. 159.

A simple modification of Russell's double sugar medium was made by introducing a semisolid layer of agar under another of the usual consistency. With this modification, motility of the organisms can be easily detected, and thus the laboratory worker is enabled to differentiate between Bacterium typhosum and Bacillus dysenteriae and between motile paratyphoid and nonmotile paracolon organisms. By substituting the use of this medium for that of the Durham sugar tubes in the preliminary identification of the colon typhoid group, at least 50 per cent of the material and labor ordinarily involved may be saved, with no decrease in efficiency.

FROM THE AUTHOR'S SUMMARY.

Society Transactions

PATHOLOGICAL SOCIETY OF PHILADELPHIA

ESMOND R. LONG, *President*

May 13, 1937

ARTHRITIS IN INFRAHUMAN PRIMATES. HERBERT FOX.

The present report is a continuation of one given at the meeting of this society in May 1936. At that time, relatively few skeletons of infrahuman primates had been examined, and these showed only moderate arthritis. Examination of more extensive material demonstrates, however, that this group of animals may show as severe forms of the disease as do Carnivora and Artiodactyla. To date 261 complete skeletons and 49 incomplete ones have been studied. The complete skeletons were from 90 gorillas, 19 chimpanzees, 15 orang-utans, 12 gibbons, 76 cercopithicidae, 26 cebidae, 20 lemurs and 3 marmosets. Among these animals were 21 which had chronic arthritis: 16 gorillas, 2 orang-utans and 3 baboons of the genus Papio. The last group, in which 19 skeletons were examined, is included in the family Cercopithicidae.

In the 21 arthritic animals both hypertrophic and ulcerative lesions occurred, and most of these lesions were seen in the spine (17 instances), where the disease involved chiefly the third and fourth cervical, the first, second, tenth, eleventh and twelfth thoracic and the third and fourth lumbar vertebrae. The anterior extremities were next in order, with lesions in the elbows and metacarpophalangeal joints. In the posterior extremity the disease occasionally involved the periacetabular tissues and the metatarsophalangeal joints. In extent and degree the changes in the anterior extremity were decidedly more advanced than those in the posterior extremity.

These observations suggest that the development of arthritis in infrahuman primates is favored by an increase of body to skeletal bulk, and that its localization is correlated with skeletal function.

PATHOLOGIC ANATOMY OF EXPERIMENTAL PURPURA. L. M. TOCANTINS and H. L. STEWART.

The clinical and pathologic manifestations of the experimental disease as produced with antiplatelet serum in the dog show a uniform evolution, the stages of which may be conveniently divided as follows: (1) acute stage (first to fifth day), showing thrombopenia, prolonged bleeding time and, in the tissues, hemorrhage, edema and deposition of pigment; (2) intermediate stage (fifth to tenth day), exhibiting a rising platelet count, short bleeding time and multiple vascular thrombi in various organs, principally in the spleen; (3) reactive stage (after the tenth day), characterized by a high blood platelet count and hyperplastic changes in the bone marrow, spleen, lymph nodes, thymus and Peyer patches of the ileum. There is some indication that these evolutionary changes are duplicated to a certain extent in thrombopenic purpura of man.

The intensity and distribution of the hemorrhages seem to be largely conditioned by the trauma and degree of stress normally undergone by the part.

Lymph vessels in the walls of blood vessels, in the bronchi of the lung and in the heart muscle, afferent lymphatics and sinuses of lymph nodes and those draining hemorrhagic areas are often distended with erythrocytes during the acute stage of purpura.

In the spleen of the animal recovering from an attack follicular hemorrhages appear when the number of platelets in the blood is higher than normal.

Megakaryocytes in the bone marrow and elsewhere undergo various qualitative and quantitative changes indicating their histogenetic relationship with blood platelets.

TRANSFORMATION OF THE VIRUS OF RABBIT FIBROMA (SHOPE) INTO THAT OF INFECTIOUS MYXOMATOSIS (SANARELLI). GEORGE PACKER BERRY.

It has been reported from this laboratory (Department of Bacteriology, University of Rochester, Rochester, N. Y.) that the virus of rabbit fibroma (Shope) can be changed apparently into that of infectious myxomatosis (Sanarelli) by inoculating rabbits with suitable mixtures of active fibroma virus and heat-inactivated myxoma virus. In the experiments which have led to this conclusion, the myxoma virus has been present in heavy suspensions of myxomatous skin lesions. In addition to myxoma virus, these crude suspensions obviously contained a host of other things. It was possible, therefore, that the "transforming agent" was derived from the infected host rather than from the virus itself. The following observations bear on this point.

Through the kindness of Dr. T. M. Rivers, my associates and I have been able to work with suspensions of washed elementary bodies of myxoma prepared in his laboratory at the Rockefeller Institute. These suspensions are essentially free from rabbit tissue and produce myxomatosis in a dilution of 10^7. In five experiments with three different elementary body suspensions, inactivated at 65 and at 75 C., we have transformed fibroma virus into myxoma virus. Adequate control observations have shown that heating these elementary bodies to 65 C. for thirty minutes renders them completely noninfectious.

The experiments reported in this note indicate that the "transforming agent" responsible for the fibroma to myxoma change comes from myxoma virus rather than from the infected host. They add strong confirmation to our interpretation that fibroma virus is actually transformed into myxoma virus.

Book Reviews

La perméabilité en physiologie et en pathologie générale. By E. Gellhorn, professeur de physiologie au Collége de médecine de Chicago, Université de l'Illinois (U. S. A.), and Jean Régnier, professeur agrégé à la Faculté de pharmacie de Paris, pharmacien-chef des Hôpitaux de Paris. Paper. Price, 160 francs. Pp. 928. Paris: Masson & Cie, 1936.

The best thing about this 7 pound (3 Kg.) volume is its beautiful printing. For the rest it is a review of what has become a now highly specialized field in biology, with an account of Gellhorn's own studies added. Originally published in Germany under the title "Das Permeabilitätsproblem," it is here translated into French by Régnier, who augments the original with criticisms and references to work and workers not appearing there. Gellhorn's views are set forth in Roman type; Régnier's additions, in italics. In length of text the two came out about equal.

The preface proposes "to make one" the problems of "the permeability of the limiting membranes of animal and vegetable cells" with those of "pediatrics, psychiatry, dermatology, ophthalmology and gynecology" (a promise hardly fulfilled, unless the reader is content to know that a woman bleeds because her uterine mucous membrane is more permeable or, in the absence of menstruation, that it is too little so).

The text proper commences with a 35 page introduction to a consideration of "methods," followed by 30 more pages on the "permeability" of "membranes"—those made in the laboratory of pyroxylin (nitrocellulose) receiving more space than those of potato skin. Forty-five pages of interpolation by Régnier are followed by 330 by Gellhorn on the "permeability" of the cell itself. These are required to "prove" what every biologist has known since Adam—that salts, acids and organic material of the most varied types do get into and out of cells. (How else, to live, could a cell gather its food materials or rid itself of its metabolic products?)

One must get through half the tome before discovering what it is all about. Then there appear very vague references to Pfeffer, de Vries, Nasse and Hamburger. Most other citations begin at 1920 (when Gellhorn himself entered the field). Unhappily the "permeability problem," to use his own words, began with the very men who created the "impermeability" features. To explain the "turgor" of plant cells—seeing that they were shrunk by stronger sugar and salt solutions and made to swell by weaker ones or water—de Vries and Pfeffer proposed the view that cells are osmotic machines. Thus they surrounded them with a "semipermeable" membrane (such as Traube had discovered in his copper ferrocyanide "precipitation" membranes) which would let water through but no dissolved substances. In testing out the adequacy of this hypothesis on living cells it should be remembered that its very authors noted a whole series of exceptions to the general rule, discovering for example that various electrolytes and nonelectrolytes passed into and out of cells as though their "semipermeable" membranes did not exist. Thus they themselves must be written down as the historic discoverers of "cell permeability." The fact made Pfeffer himself emphasize the need to look for mechanisms which would allow the living unit to take up and give off water while it was at the same time taking up and giving off dissolved substance. But no one paid any attention to his warnings. Thus thirty years passed, with most biologic workers holding to the impermeability of cell membranes. One school, for example, maintained that all salts or all chlorides could not enter living cells—a belief, however, never put to the practical test of eating mercury bichloride on lettuce in lieu of common table salt by any of its advocates. The reason for holding so tenaciously to the notion of semipermeability lay in the need of explaining the movement of water into and out of cells, for in pro-

portion to their discovery of a dissolved substance in the "permeable" class, differences in concentration between the inside and the outside of cells disappeared; and so the necessary "force" to move it. The more modern "permeabilists," as witness this volume of Gellhorn, have now carried the matter so far that they just ignore this phase of the total problem.

Having thus witnessed all manner of changes in "permeability" of "the limiting membrane of living cells" (the simple physical and biologic concept of the original "osmotic" layer changes quickly to the "lipoid" and is soon thereafter inextricably mixed up with "plasmatic," "mosaic" or even "surface tension," "interfacial" and "adsorption" notions of such cellular envelops), the author proceeds to 235 pages on the "permeability" of organs. That finding "permeability" is just another way of saying that the intestinal tube, the serous cavities and the tissues themselves have a way (in the higher animals) of absorbing what may be squirted into them, or that the kidney, skin or lung has a way of secreting these same substances, is nowhere made clear. Things just get more "permeable"—or less so if another author is cited—and that is all there is to it.

Not until page 769 is reached is the question raised whether, perhaps, there are no limiting membranes at all or whether the membranes conceived of are not limited to a cell covering but run "clean through" the works, whether, in other words, the totality of "protoplasm" is "membrane" (including the intercellular substances and such circulating fluids as the blood and lymph of higher animals). Four authors are quoted as of such a view—but what are four against a 50 page list (approximately 1,500 citations, not counting Régnier's additions) who say to the contrary?

The strongest feature of Gellhorn's volume thus comes to reside in the number of his reviews of other men's work; he himself exercises small critique and little evidence of choice of scientific fact or of the weight to be placed on it. The book carries no index, but rather careful reading showed that Kite's (1912) first microdissections of the cell are nowhere mentioned, nor Kahlenberg's (1898) studies of osmosis and the inapplicability of the ionic dissociation concept in aqueous solution to protoplasm. Hofmeister is quoted twice, his pupil Spiro four times and Pauli not at all. The younger Ostwald also appears to be cited nowhere, not to mention a score of lesser workers in the colloid field who have known for decades that if laboratory membranes and those assumed to be existent about cells or to make up their entire structure have anything at all in common, it centers entirely about their (hydrophilic) colloid make-up.

Gellhorn called his German text the *problem* of permeability; the French still leaves it this problem.

MARTIN H. FISCHER.

Surgical Pathology of the Thyroid Gland. By Arthur E. Hertzler, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kan., and Professor of Surgery, University of Kansas. Cloth. Price, $5. Pp. 298, with 238 illustrations. Philadelphia: J. B. Lippincott Company, 1936.

This is the latest of eight monographs on surgical pathology by the author. The monograph is in many ways unusual. Even without knowledge of the personality of the author, the reader will recognize at once that the book is the outcome of a rare combination of long clinical and surgical experience with interest in and knowledge of gross as well as microscopic pathologic pictures. It is obvious that the author has made the best of his opportunity of watching his patients over long periods of time; he has observed signs and symptoms from their earliest stages and has looked at the removed thyroid glands and at the histologic sections with the understanding of an experienced and interested pathologist; he has followed up his patients and checked in that way the effects of the operations.

The book has a distinctive personal flavor. The approach of its author to some highly controversial questions is never orthodox and sometimes not entirely borne out by established facts, but it is always interesting and thought provoking. All through the book runs its basic idea, like the leitmotif of a musical composition.

It will be best expressed in the author's own words: "Lest I forget to do so, I may say at the outset the idea is to emphasize the fact that goiter, whatever the type in the beginning, is a continuous process ending in a cardiotoxic state." One might question the scientific accuracy of this concept, and no doubt many would oppose it bitterly and with strong arguments.

According to Hertzler, the degenerative changes in the thyroid gland, in the parenchyma as well as in the connective tissue, exert a profound toxic influence on the body, particularly on the heart, and their effect is distinct and apart from the hyperplastic changes. That, too, may not be accepted as proved. However, all these objections represent merely different points of view. The same holds true for the classification, except that it is quite difficult to see a justification for the designation "mixed tumors" for growths that are microscopically malignant and clinically benign. It is not apparent from the text, or from any other source, on what basis Hertzler claims that such tumors resemble pathologically the mixed tumors of the parotid gland. Serious objections must be raised against Hertzler's designation, since without any good reason it conflicts with the accepted and well founded concept of mixed tumors.

The style is pleasing and effortless. Many humorous remarks and phrases add to the reader's enjoyment. There are thirteen chapters. In the first chapter a general classification is presented. The normal morphology, the general gross pathology and the microscopic pathology are discussed in the following chapters, and after that come chapters devoted to the presentation of the special pathology of the nontoxic diffuse goiters and of nodular goiters, of the toxic nodular goiter, of the cardiotoxic goiter, of the atypical toxic goiter, of the toxic diffuse goiter, of thyroiditis, of myxedema, of fetal adenoma and of tumors. The bibliographic references are scanty. The index fills 18 pages and is well organized. The illustrations are of uniformly high grade. The paper, print and binding are excellent.

The Endocrines in Obstetrics and Gynecology. Raphael Kurzrok, Ph.D., M.D., Associate in Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University. Cloth. Price, $7.50. Pp. 500, with 178 illustrations. Baltimore: Williams & Wilkins Company, 1937.

In this volume the newer physiology of each of the endocrine glands and of their various interrelationships is briefly reviewed. The author attempts to apply this knowledge to the diagnosis and treatment of disorders of the sex functions in obstetrical and gynecological practice. A number of supplementary chapters on the history, embryology and chemistry of the "sex hormones," etc., are also included. The text is systematically arranged and well illustrated and includes illustrative case histories. A bibliography accompanies each chapter; a subject and author index completes the book.

The author's courage in attempting such a book at this time is commendable. The recognition of the extent to which the book falls short of its avowed clinical purpose should be tempered by a realization of the recent and incomplete nature of much of the underlying physiologic investigation. A convenient collection of the basic experimental facts, which particularly concern the obstetrician and gynecologist, is indeed presented. But the prospective reader must be prepared to find that the amount of this material which can be clinically applied at the present time is relatively slim. And much of that which does have apparent clinical application can often be used only in a trial and error manner. All in all, this book may constitute a useful summary for the physician who has been following the development of the subject, but it will not make an endocrinologist out of the neophyte.